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THE EFFECT OF TESTOSTERONE PROPIONATE ON THE GENITALIA, PROSTATE, SECONDARY SEX CHARACTERS, AND BODY WEIGHT IN EUNUCHOIDISM[1]

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With the synthesis of testosterone from cholesterol by Butenandt and Hanisch (1), and by Ruzicka and Wettstein (2), and the successful large-scale commercial application of their processes, it is at last possible to undertake substitution for the testis in man. Although not as yet established as the actual hormone, testosterone has been obtained from bull testis by David, Dingemanse, Freud and Laqueur (3) and, with the exception of certain of its own esters, is the most potent of known androgens. It will repair castration defects practically completely in male animals. The literature dealing with this evidence has been reviewed recently by Koch (4) and need not be dealt with here.

In 1936, Miescher, Wettstein and Tschopp (5) discovered that various fatty acid esters of testosterone not uncommonly stimulated the prostate and seminal vesicles of the rat more than did testosterone itself. This was true especially of the formate, acetate, propionate and butyrate, and not at all of the palmitate and stearate which were inactive. Curiously enough, no such enhanced value from esterification was apparent when the growth of the capon's comb served as the test, although certain compounds, as the butyrate, had a more prolonged action than testosterone itself. The bearing of these facts on the manner in which the hormone is secreted is not clear. Esterification may well influence merely the rate of absorption from the depots in the skin, as appears to be the case when fatty acids are injected with testosterone (6). Parkes (7) has pointed out that, although dividing the dose increases the activity of testosterone, such is not true of the acetate. This is in keeping with the view that the difference between the two substances lies in the more gradual absorption of the ester, so that concentrations in the blood favorable

[1] A preliminary report of this study has been published (16).

for destruction and excretion are not so easily reached. The synergistic effect of the lipoid soluble x-substances of the Laqueur school (3, 8) may also reside in just this property of retarding absorption from the skin, and any physiological significance they have, therefore, would depend on the extent to which absorption from the testis itself into the blood stream was so influenced. As the qualitative effects of the esters in nowise differ from those of testosterone, it appears proper to set aside for the time being the question as to which more nearly represents the actual hormone, and to use such an ester as the propionate in biological and clinical work, taking advantage of the fact that it is some 4 times more powerful in stimulating the accessories than is testosterone itself.

We are concerned in this paper with the treatment of eunuchoidism, in which the symptoms are those of testicular deficiency. The testes are retained in the abdomen or, if in the scrotum, are small. The penis and prostate are hypoplastic. Erections usually occur but ejaculation is absent or scant, with few if any sperm. Pubic, axillary, limb, facial and trunk hair are scant and the fine skin, smooth and boyish in the young, becomes greatly wrinkled with age. The voice is variable in pitch, sometimes quite high, more often within the upper range of normal, rarely if ever deep, and the laryngeal prominence is small or absent. Many epiphyses that normally fuse with their metaphyses after puberty may be found ununited if the patient is young, but in time union does occur. Characteristically, the arms and legs are relatively long. The patients are no heavier and no lighter than others, but local accumulations of fat over the pubis and hips and in the breasts may be present. When the patient is heavy, the designation of Fröhlich's syndrome is often applied, but it may be questioned whether any useful purpose is served by assigning the fat to a separate category, when all grades of adiposity and its lack are found in the hypogonad. Dr. Hugh Carmichael and the author (9) have reviewed the mental changes in these people, finding that sexual desire is often developed to some extent at least, and that no specific personality pattern exists. They are not effeminate, nor are they often homosexual. The excretion of androgens in the urine has, in our experience, averaged about a third of that in the normal, but the values for low normal may be overlapped (10). Estrogens are likewise diminished. Metabolic peculiarities are not obvious nor profound, and will be discussed in a subsequent paper. It is usually not possible to determine whether the defect is of pituitary or of gonadal origin; large amounts of gonadotropic material have not been found in the urine as in the castrate. Even careful necropsies have often left considerable doubt as to the rôle of the anterior lobe of the pituitary (11, 12), but on occasion gross lesions as suprasellar cysts are responsible. Treatment of the adult with pregnancy urine preparations has been unsatisfactory as a rule. Striking improvement may be produced, but good results seem as yet to be less common than in the hypogonad boy (9).

In deciding upon the dose of testosterone to be used in treatment of eunuchoidism, advantage may be taken of the fact that the average normal excretion of androgens in the urine in young men amounts to about 70 I.U. daily (13). This represents the activity of 7 mg. of androsterone, or 21 mg. of dehydroisoandrosterone, both of which substances are present in normal urine. Assuming that for each molecule of these substances a molecule of

testosterone is metabolized, from 7 to 21 mg. of testosterone are altered and lost daily in the urine. The probabilities are that this urinary loss represents only a fraction of the total testosterone disposed of by the organism. The daily dose of 25 mg. testosterone propionate which we have selected in most of our work is therefore not unreasonably high. The experiences of Dr. Carl Moore (15) with the quantities of testosterone propionate required to replace exactly the activity of the testis in the growing rat likewise suggest large amounts should be used clinically. Our own unsatisfactory preliminary experience with testosterone itself in doses of 2.5 mg. was therefore not surprising.

Our experience with the prolonged treatment of 4 eunuchoids with testosterone and testosterone propionate[2] is given in detail in the following proctocols.

PROTOCOLS

J. H., a eunuchoid, aged 36 years, had bilateral cryptorchism which had been operated on unsuccessfully. His pubic, axillary and limb hair was scant, and facial fuzz was shaved every 2 weeks; his voice was a somewhat high-pitched masculine. His penis was small, measuring 4.1 cm. long and 2.3 cm. wide at the corona. The prostate gland could not be felt. He had noted erections, but never seminal discharge. He was married and was able to satisfy his wife in intercourse. He was 180.6 cm. tall, and his greatest arm stretch was 198.7 cm. (110 per cent of stature) as compared to the average normal of 102.6 per cent. His sitting height was 91.1 cm., giving a sitting:standing ratio of 50.4 as compared to the average normal of 52.9 [Hrdlička (17) should be consulted for a discussion of anthropometric norms]. The arms and legs are thus disproportionately long. X-rays showed incomplete fusion of the epiphyses of the proximal ends of the humerus and tibia and distal end of the radius, which normally fuse with their metaphyses by the age of 25 [for the standards Hodges' chart (18) was used]. The sella turcica and the visual fields studied for form were not abnormal. His B.M.R. was −19, Mayo Foundation (19), and the glucose tolerance was normal. A 3-day urine collection showed the presence of 33 international androgen units per day, which is within the lower normal range.

From October 12th to December 29, 1936, the patient took 39 cc. of testosterone in sesame oil, and 2.5 mg. of testosterone per cc. intramuscularly, a total of 97.5 mg. in 76 days. He received 1 cc. practically daily during the last month. There was an immediate increase in erections and in sexual tension. During the second month the penis elongated to 5.8 cm., but the prostate was not altered at all. Small flat buttons of breast tissue appeared under each nipple. On March 24, 1937, the penis again measured 4.1 cm. long.

Between March 31, 1937, and July 8, 1937 (99 days) he received subcutaneously 86 cc. of testosterone propionate in sesame oil, 25 mg. per cc. During this time, he took 2150 mg. in all. During the first 65 days he took 1 cc. 7 times weekly, during the last 34 days, 1 cc. 5 times weekly.

Within 16 hours after starting the testosterone propionate, he was aware of greatly increased frequency of erections. This progressed until the erections became nearly constant, and a 'cramping' sensation was felt at the root of the penis. Some soreness of the breasts appeared on the 10th day, and on the 12th his penis was 6.0 cm. long, an increase of 50 per cent. The prostate could be defined on the 12th day,

[2] We are indebted to Drs. Erwin Schwenk and Gregory Stragnell of the Schering Corporation for the large amounts of testosterone and testosterone propionate used.

and was unmistakably enlarged on the 27th day. Slight huskiness of the voice appeared by the 27th day, and progressed until, at the end of 95 days, friends commented upon the deeper pitch. On the 25th day, a small ejaculation occurred during intercourse and was present thereafter. By the 42nd day nubbins of breast tissue 3 x 2.7 cm. in diameter were palpable under each nipple. By this time (day 42) the erections had subsided somewhat in frequency, and the cramping sensation had disappeared. The penis did not increase further in size but remained between 5.3–6.2 cm. long. The prostate, however, continued to enlarge until each division palpable was between a nickel and a quarter in area. On the 65th day a little clear fluid could be expressed from the nipples. By the 95th day a definite increase in pubic hair could be detected, and hair could be seen up the linea alba. A little terminal hair appeared on the upper lip.

On July 7, 1937 (day 99) he was placed on testosterone propionate, 25 mg. 3 times weekly, and on September 10 (day 163), he was changed to the same dose twice weekly. By day 163, the voice was maintained at an even low pitch and the body hair had increased up the linea alba, over the thighs and on the chest. He shaved twice weekly as compared to every 2 weeks before treatment started. Intercourse, practiced every 2 to 3 weeks before, now took place every other day, and he would have preferred it daily. The breast enlargement subsided. On day 221 (November 7) there was no further increase in the signs or symptoms of puberty; indeed the sexual stimulation was substantially below that on the previous high dosage level. The total dose on November 7 was 3375 mg.

The striking increase in body weight is shown in figure 3. During the period of weight gain, the patient reported that his appetite was much greater than normal. His face and hands became full and puffy. There was no pitting edema of the extremities. With the reduction in dose on day 99, loss of 5.2 kg. in weight occurred, until on day 163 he weighed 85 kg., at which point he remained.

H. K., a eunuchoid aged 24 years, had a bilateral cryptorchism which had been operated on unsuccessfully. His pubic, axillary, limb and trunk hair was scant, and he had not shaved. His voice was masculine but not of low pitch. His penis was 3.6 cm. long and 1.6 cm. wide at the corona. The prostate gland was not palpable. He had noted erections and had been able to perform intercourse but had had no seminal discharge. He was 168.9 cm. tall, and his span (181.3 cm.) was 107.4% of his stature. His sitting height was 86.5 cm., giving a sitting:standing ratio of 51.2 as compared to the average normal of 52.9. The arms and legs were therefore relatively long. X-rays showed epiphyses of the head of the humerus, coracoid, proximal ends of the tibia and fibula, proximal and distal ends of the radius and distal end of the ulna to be incompletely fused with their metaphyses. The delay here is from a slight one to as much as 6 years. The sella turica and the visual fields studied for form were not abnormal. His B.M.R. ranged from −5 to −19 (Mayo Foundation standards). His glucose tolerance showed the curve of a mild diabetes mellitus. He excreted 18 international androgen units per day in his urine, and equivalents of 1 to 2 gamma of theelin, both values being low.

From June 29, 1936, to October 30, 1936, he took 71 cc. of testosterone in sesame oil, 2.5 mg. cc. subcutaneously, a total of 177.5 mg. in 123 days; 1 cc. approximately 5 times weekly during the last 2 months. By July 12th a slight increase in the frequency of erections occurred, and by September 3rd a slight questionable increase in prostatic tissue was palpable. The penis increased in length to 4.6 cm. From November 20, 1936 to December 24, 1936 he took 25 cc. of testosterone propionate in sesame oil, each cc. containing 5.0 mg., a total of 125 mg. The penis

increased to 5.8 cm. in length, but there was no change in the prostate. In February 1937 the penis was still 5.7 cm. long.

Between February 17, 1937 and April 22, 1937 (64 days) he received 53 cc. of testosterone propionate in sesame oil, subcutaneously, 25 mg. per cc. In all he took during this time 1325 mg., administering the injections daily with occasional brief interruptions.

Within 18 hours after the first injection he noted an increase in the frequency of erections, with increased sexual desire. At times the erections became nearly constant. By the 17th day there was a small amount of prostatic tissue palpable. The penis became slightly larger, and measured from 6.5 to 7.3 cm. long and 2.5 cm.

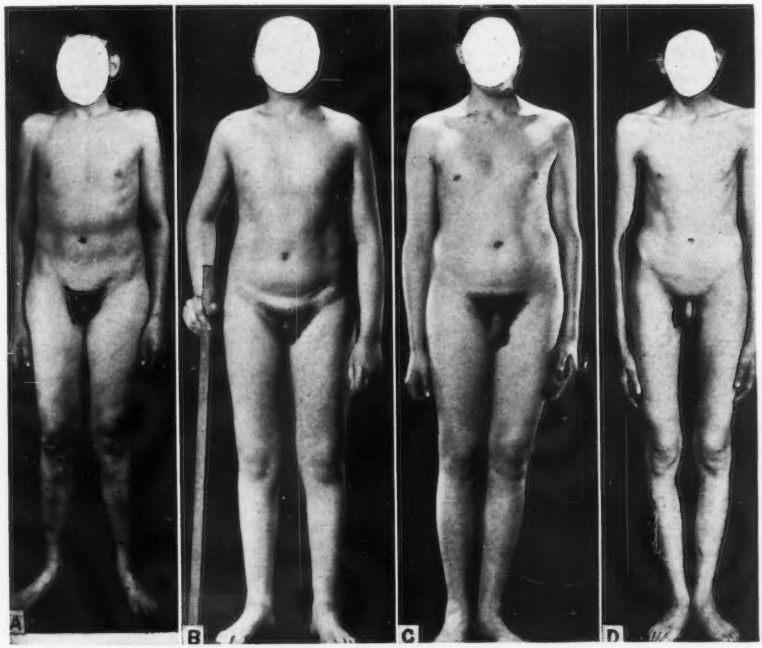

Fig. 1. Anterior views, *A*, of *J. H.*; *B*, of *H. K.*, *C*, of *N. T.*, and *D*, of *N. D.*

wide between the 26th and 64th days. On the 36th day the prostate was clearly felt as a soft mass with the area of a nickel, and was further enlarged by the 64th day. His voice was noted as husky on the 26th day, and by the 36th day the patient's brother had commented on its depth. A growth of terminal hair appeared at the outer corner of the upper lip by the 36th day, on the chin by the 50th day and in the burnside area by the 64th day. From then on the extension of hair in the pubic area, over the thighs, and onto the abdomen was apparent. Because of ill-defined and unexplained precordial discomfort the testosterone propionate was discontinued on the 65th day. For a week after stopping, the sexual excitability continued and then receded. Eighteen days after stopping treatment the prostate was soft and could barely be defined. The hair growth did not recede and his voice remained deep. Ten mg. of testosterone propionate was given daily for 10 days beginning on May

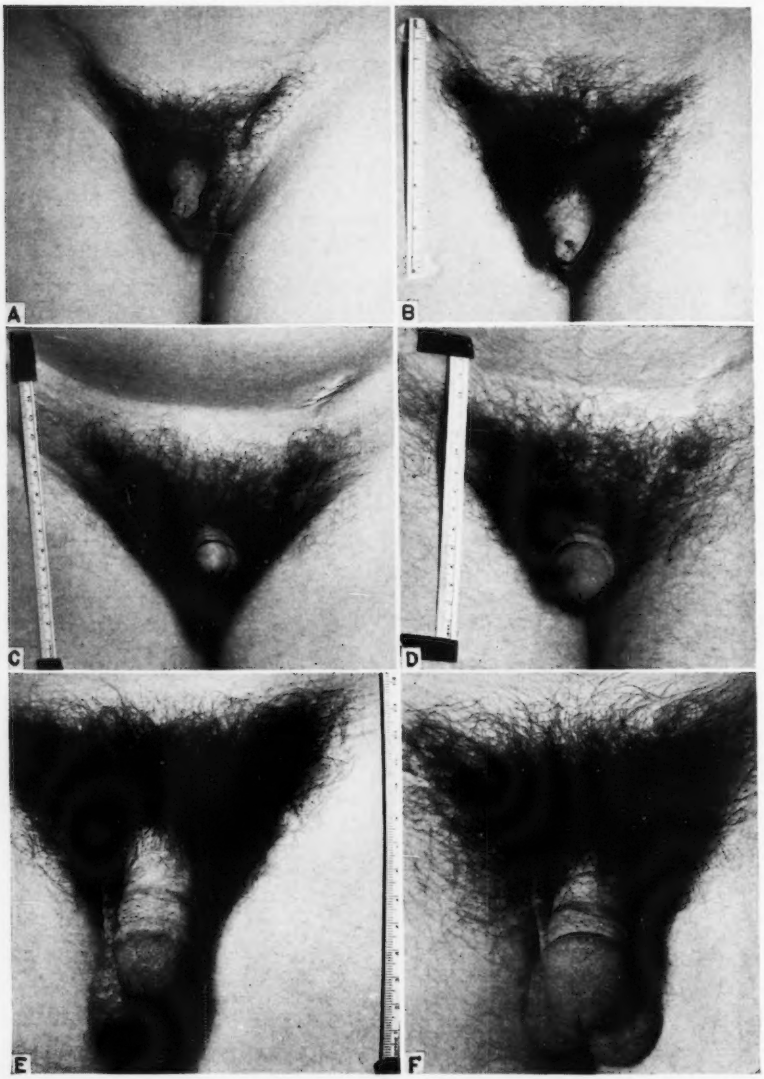

Fig. 2. *A*, the genitalia of *J. H.* before, and *B*, after 163 days of treatment with testosterone propionate; *C*, of *H. K.* before and, *D*, after 111 days of treatment; *E*, of *N. T.* before and, *F*, after 181 days of treatment. The increased hair growth in *N. T.*, which is much less striking than in the other 2 shown, is definite on close inspection although not disclosed here. The scale of the photograph on the right (*F*) exceeds that on the left (*E*) by 5 per cent.

26, 1937, but the precordial discomfort again returned. Erections were stimulated by this dose, and a little clear fluid could be expressed from the nipples at this time although there were no palpable changes in the breasts. On July 16th the 10 mg. dose was again started 3 times weekly, but even so the precordial discomfort became so trying that treatment was again stopped after 23 injections, the last on September

20th. The length of the penis and the size of the prostate remained unaltered. The penis was 5.4 cm. long on September 30th, the same as before treatment with the 25 mg. dose.

The precordial discomfort of this patient was exceptional. It was continuous, aching in character, accompanied by some precordial tenderness and not intensified by exertion. The origin and significance of this pain were obscure, but it appeared with each of three courses of testosterone propionate and ceased thereafter. No changes in the size, rate, rhythm or tones of the heart could be detected, the blood pressure was undisturbed and no conclusive electrocardiographic abnormalities ap-

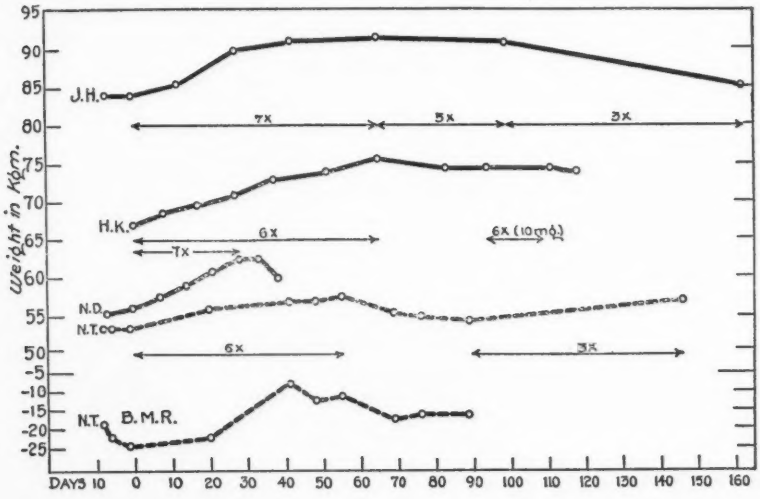

Fig. 3. The effect of testosterone propionate on body weight of 4 eunuchoids and on the B.M.R. of one (*N. T.*), the diet and activity being uncontrolled. The numbers followed by x indicate the number of times testosterone propionate was injected weekly, the dosage being 25 mg., except where indicated.

peared, although the P wave was low in the three standard leads, S-T II was very slightly elevated and T II was high in amplitude. Lead IV was normal.

The substantial increase in body weight is shown in figure 3. His appetite became so greatly increased that he was unable to endure the conventional distance between meals. While his face became puffy, pitting edema of the legs was not shown. He complained frequently during the treatment of flushing of the face and of transient sensations of heat.

N. T., a eunuchoid aged 30, had previously been treated with several different preparations. Our experiences with bull-testicle extract and androstanediol have been briefly reported (20). From January to October 1936 he received a pregnancy urine extract, and during this time remarkable advances in sexual development had occurred (9). At the time of starting testosterone propionate in April 1937 further progress was at an end, but he was more advanced than the first two men recorded. He had a fair amount of pubic and axillary hair and shaved fine terminal lip hair every 2 weeks. His voice was of low pitch. His penis was 7.1 cm. long and 2.8 cm. wide at the corona. Six examinations of ejaculates during the preceding 5 months had consistently shown the presence of about 2 motile sperm per high power field. His testes measured 3.6 x 2.2 x 2.0 cm. on repeated measurements. Each palpable

prostatic division was estimated as 1.5 cm. long and 7 mm. broad. He was 163 cm. tall and his sitting height 80.7 cm. (sitting:standing ratio = 49.0) and his span 172 cm. (span:stature ratio = 105.5) showed evidence of the eunuchoid disproportion. The epiphyses about the shoulder, knee, wrist and hand which showed delayed fusion in 1933 had all fused by April 1937. The sella turcica and the visual fields were not abnormal. His B.M.R. was −19, −22, and −24 by the Mayo Foundation standards, his glucose tolerance was normal. The most recent assay showed 15 international androgen units per day in the urine and the equivalent of one gamma of theelin.

Between April 25, 1937 and June 18, 1937 (54 days) he received 43 cc. of testosterone propionate in sesame oil, 25 mg. per cc. subcutaneously. In all during this time he took 1075 mg., receiving an injection 6 times weekly. Some stimulation of sexual desire and some increase in frequency of erections occurred, but these were not as striking as he had observed the year before, when he took pregnancy urine extract. On the 20th day the penis was recorded as 8.1 cm. long, but thereafter it measured 7.2 and 7.4 cm. as at the beginning. The prostate was larger by the 20th day and by the 46th day was nearly of normal size. Examination of the semen for sperm showed a few dead sperm on the 20th and 34th days, and none on the 48th. A slight increase in hair up the linea alba was noted on the 54th day. On the 34th day many pimples appeared on the face, just as they had during treatment with pregnancy urine extract. Between June 19th and July 24th no treatment was given. Testosterone propionate was then given in 25 mg. doses, 3 times weekly until September 18th. By this date (day 146) a definitely increased hair growth was present on the lips, chin, cheeks, legs, arms and abdomen. The testes were measured frequently, but at no time changed in size or in consistency. From September 18, 1937 to December 1st, one 25 mg. dose was given weekly. On January 6, 1938 one motile and one nonmotile sperm were again found per high power field.

The changes in weight and in the B.M.R. are shown in figure 3. The patient developed some fullness in the face during the period of weight gain, but he did not notice any considerable increase in appetite, nor did he exhibit any obvious edema of the extremities. He noted the flushing of the face and the warmth, as described by *H. K.*

N. D., a eunuchoid aged 31, had been treated with PU extract between January 7th and July 7, 1936 with resulting slight enlargement of the genitalia but very little else (9). His pubic and axillary hair was scant, his limbs and face smooth and his voice a slightly high-pitched masculine. His penis was 7.1 cm. long and 2.7 wide at the corona. The testes measured 2.5 x 1.6 x 1.4 cm. The prostate was composed of a pea-sized left lobe and a soft ill-defined right lobe. Erections occurred but no ejaculations. He was 175.7 cm. tall, his sitting height, 83 cm., giving a sitting:standing ratio of 47.2 cm.; his span, 190.5 cm. gave a span:stature ratio of 108.4, these relations showing a striking eunuchoid disproportion. The epiphyses of the head of the humerus, of the distal ends of the radius, ulna and femur and of the proximal ends of the tibia and fibula and of the crest of the ilium were incompletely fused, a delay of at least 5 years. The sella turcica and visual fields were normal. His B.M.R. ranged from −7 at first to −33 later (Mayo Foundation standards). Fasting blood cholesterol was 155 mg. per 100 cc. His glucose tolerance was normal. He excreted 7 international androgen units per day in the urine and the equivalent of 1 γ of theelin.

Between November 17, 1936 and December 11, 1936 he received 22 cc. of testosterone in sesame oil, subcutaneously, 2.5 mg. per cc., in all 55 mg. There was

no change in the size of the penis or in the prostate. On February 10th he weighed 123¼ lb. (56 kg.), which was about his weight for the preceding year.

This patient was started on testosterone propionate, 25 mg. daily, on February 10, 1937 and continued until March 10th, receiving in all 28 cc. (690 mg.), by subcutaneous injections. His penis elongated to 8.5 by the 7th day, and remained between 8.5 and 8.9 cm. during the course of treatment. There was a moderate increase in the frequency of erections by the 3rd day, but this was never emphasized by the patient. By the 14th day the prostate was recorded as definitely larger. By the 20th day the left lobe of the prostate had an area the size of a nickel, and the right lobe which had been very thin was definitely fuller. His voice was recorded as deeper. The testes, which were measured frequently, did not change in size or in consistency. Because of the patient's departure for abroad, treatment was discontinued. On his return, several months later, the prostate was again very small and his penis was 7.2 cm. long. The changes in weight are shown in figure 5. Fullness of the face and pitting edema of the legs developed with the gain in weight, but no increase in appetite was noted.

SUMMARY OF RESULTS

Three eunuchoids (*J. H., H. K., N. D.*) received 97.5, 177.5 and 55 mg. of testosterone, in 2.5 mg. doses, in 76, 123 and 24 days. In two (*J. H., H. K.*) there was an increase in erections and an elongation of one centimeter or more in the penis, and in one (*J. H.*) the development of small buttons of breast tissue, but in no instance did prostatic tissue increase clearly in amount. In one (*H. K.*), 125 mg. of testosterone propionate in 5 mg. doses in 34 days was given with the production of increased erections and further elongation of the penis by one centimeter, but again no change appeared in the prostate. The absence of prostatic change together with theoretical considerations of the proper dosage led us to proceed with larger amounts of testosterone propionate.

Four eunuchoids received 25 mg. of testosterone propionate, subcutaneously, from 5 to 7 times weekly for 28 to 99 days. Thereafter, in 3, from 10 to 25 mg., 3 to 7 times weekly, was given with interruptions until the 146th, 108th and 163rd days respectively. In all there was an early increase in the frequency of erections and a marked increase in the size of the prostate (day 12 to 36) with recession after discontinuance in the 2 studied. The penis elongated from 1 to 2 centimeters in all but one (*N. T.*), but this enlargement did not progressively increase during the course of treatment. Enlargement of the scrotum was not especially striking. In 3 (*J. H., H. K., N. D.*) there was a distinct deepening of the voice (day 27-95, 26-36, 28) and the exception (*N. T.*) had a fairly deep voice to begin with. In two (*J. H., H. K.*) there was a striking development of hair in the pubic area, up the linea alba, over the thighs and on the face (days 95 and 64 on), whereas in *N. T.*, who was further advanced to begin with, the hair increase although distinct was more moderate (day 54 on), and the photographs show it poorly or not all (figure 2). *N. D.*, who was treated for 28 days, showed no change in hair growth.[3] Recession in the size of the penis and of the prostate after stopping treatment occurred in the 2 (*N. D., H. K.*), in which opportunities for such observations were provided. In *J. H.*, nubbins of breast tissue, 3 x 2.5 cm. in diameter, appeared under the nipples and in *J. K.* and *H. K.* a slightly opalescent fluid could be expressed therefrom. In the 2 cryptorchid patients the testes did not descend, but in

[3] The succeeding paper (39) provides a description of the prostatic enlargement occurring in 4 eunuchoids treated in the hospital. Confirmation of the results reported here on secondary sex characters has been since provided by observations on two of three more eunuchoids treated for long periods.

view of the scars of previous surgery the integrity of the tissue in and about the inguinal canals had been destroyed. The 2 (*N. T., N. D.*) with testes in the scrotum showed no changes whatever in either the size or consistency of these organs, although careful measurements were made. In one (*N. T.*), the few sperm, which had been present in the semen consistently for 5 months, disappeared during treatment and reappeared 1 month after discontinuance. An acniform eruption appeared on the face of one patient (*N. T.*) Libido was increased in three.

Remarkable and, at the time, completely unexpected was the consistent and considerable gain in body weight which is shown in figure 3. In the 4 patients recorded here, the gain in 10 days was 1.5 kg. in 3 and 2.0 in *N. D.* The maximum gain of 4.5 to 8.5 kg. was reached in 28 to 65 days. Only in *J. H.* and *N. T.* was treatment continued after this high point, and in both a plateau was established. Fullness and puffiness of the face was present in all, puffiness of the hands in *J. H.* and definite pitting edema of the legs in *N. D.* In 2, *J. H.* and *H. K.*, the appetite was greatly stimulated. Reduction of the dose of testosterone propionate led to a loss in weight in *J. H.*, discontinuance to a loss in 2 (*N. T., N. D.*), but in *H. K.* in which the edema was not obvious a loss of 1 kg. only occurred in 29 days after stopping treatment. The B.M.R. determinations on *N. T.* (fig. 3), showing if anything a slight rise, indicate that lowering of the basal caloric requirements plays no part in the weight gain.

DISCUSSION

Concurrently with our own, several other studies of the effects of testosterone and its esters in man have been made, and such as are known to us will be briefly reviewed here. Moricard and Bize (21) treated 10 boys from 5 to 14 years of age presenting adiposo-genital dystrophy, anomalies of the testes as inequality and ectopic position with slight obesity, obesity, infantilism (genital retardation associated with undernutrition and short stature) and enuresis with testosterone acetate, giving from 50 mg. in 1½ months to 570 mg. in 6 months. There was in all an elongation of the penis of from 1 to 2 cm. and an increase in its circumference, in several a questionable decrease in the size of the testes, in several a considerable gain in body weight, to the extent of 4 kg. in 2½ months (Am) and 5.3 kg. in 6 months (Rovil). In one, an 8½ year-old boy, said to have adiposo-genital dystrophy, pubic hair appeared about the root of the penis in 3½ months after 420 mg. of testosterone acetate. Transformations in the distribution of body fat and gains in stature are suggested, but the data are not conclusive on these points.

Bauer and Koch (22) obtained, in a 26-year-old eunuchoid, increase in erections, enlargement of the penis, ejaculations and descent of the testes, with a questionable increase of hair on the face and extremities after 20 injections, each containing 5 mg. of testosterone propionate given every other day. Four impotent men, one a castrate, one a patient with atrophy of the testes and gynecomastia, responded with increased erections and restored potency, after similar dosages, but the authors note failure in others, including a 17-year-old boy with 'universal infantilism.'

Schmitz (23) treated 42 older men with testosterone propionate in 5 mg. doses and reported in 36 improvement in sense of well being, return in sexual desire, nocturnal emissions, etc. This study does not emphasize nor record objective changes.

Hamilton (24) treated a typical eunuchoid, aged 27, with 550 mg. of testosterone acetate in 14 injections, 3 injections per week for 1 month, and reported increased erections to the point of priapism, enlargement of the penis and scrotum, descent of 2 previously undescended testes, increase in size of the prostate, questionable lowering of the voice, appearance of hair on the chest and over the pubis, together with an increase in hair growth on the upper lip, and the proliferation of subareolar breast tissue containing lactiferous ducts and connective tissue but no alveoli. Peanut oil alone had no effect whatever. An acniform eruption appeared after withdrawal of treatment. Hamilton has also reported stimulation of erections in boys and in an impotent man.

Dr. L. E. Holt, Jr. (quoted by Young, 25), treated a 2½-year-old boy with papilloma of the larynx with daily injections of testosterone (dose not given) for 10 months, with a great increase in the size of the penis and in the frequency of erections, but with no palpable increase in the size of the prostate, nor of the testes, no growth of hair and no changes in the epiphyses of the bones or in growth in general. Foss (26) gave a 38-year-old eunuch 940 mg. of testosterone acetate in 12 weeks, with resulting increase in erections to the point of priapism, restoration of potency and a gain in weight of 16 lb.

Although no attempt will be made to review the work done with impure androgens, because of the general lack of conclusive results, note should be made of the account of Usui and collaborators (27), who reported that a urinary androgen preparation given to 3 aged eunuchoids produced increase in appetite, elevation of the B.M.R. (amount not stated), disappearance of skin folds and increases in body weight of 3.2 to 6.7 kg., in 55 to 90 days, together with restoration of erectile power in 2 and increases in the number of red blood cells and hemoglobin.

The neurocirculatory mechanism for the erection of the penis seems in the hypogonad to be most sensitive to increases in the male hormone content of the body. This augmented irritability appears often within 24 hours of the initial injection of 25 mg. of testosterone propionate, and was manifest in our earlier experiences with bull-testis extract, androstanediol (19), testosterone (2.5 mg.) and testosterone propionate (5.0 mg.), none of which altered the prostate, and has been reported by Bauer and Koch (22) and by Hamilton (24) in eunuchoids, and by Bauer and Koch (22) and by Foss (26) in eunuchs, as well as in children (24, 25) and in certain impotent men (22, 23, 24). As we have directed our full attention to the production of objective changes, we have not instituted the rigid control with placebos which the subject requires. Hamilton's eunuchoid, however, could distinguish between testosterone acetate containing peanut oil and plain peanut oil, and this together with the effects in children and the regularity of the observations in adults suggests very strongly that the androgens do really increase frequency of erection in certain conditions in man. The physiological significance of this remains as yet obscure.

The 4 following possibilities suggest themselves, and only most carefully controlled work will disentangle them. *a)*, The erectile power of the penis in man is developed and maintained by the activity of the nervous system, and

the male hormone acts in a supplementary fashion to excite this mechanism further, or to vary the threshold to psychic stimulation. *b)*, The nervous mechanism is entirely dependent upon the male hormone for its development and maintenance. This view requires the assumption that the very small amounts of male hormone circulating in the infant suffice, and that androgens of nongonadal origin so operate in certain castrates, maintaining their potency. *c)*, The mechanism is entirely nervous, and the apparent influence of hormones in our experiments is of no physiological significance, but rather to be interpreted as due to reflex stimulation from the penis, which is enlarging at an abnormal rate, together with the psychic receptivity of the patients. *d)*, The nervous mechanism is matured by hormonal influence, and may thereafter operate undisturbed in the absence of hormones.

Enlargement of the penis which, in our experience, occurred early in treatment as a rule, was not progressive to the point of forming a hypernormal organ. The slightness of the change on the whole was probably due to the fact that the organ was not in any instance extremely hypoplastic to begin with. This change, together with the enlargement of the prostate, deepening of the voice and increased growth of body hair are of course to be expected once the proper dosage is found, in view of the well-known effects of testosterone and its esters in animals. The minimal dose level required to produce these changes is as yet uncertain, but it is of interest that *H. K.*, whose prostate enlarged readily on 25 mg. of testosterone propionate daily, showed no change in this respect on 10 mg., 3 times weekly, or on 5 mg., 6 times weekly. It should also be recalled in considering dosage that from 7 to 21 mg. of testosterone must be metabolized daily in the normal man to account for the androgens in the urine, and that this amount probably constitutes only a fraction of that destroyed in the body. It is probable, therefore, that to secure conclusive results in the eunuchoid in a reasonable period of time one will not be able to go much below the dosage outlined here.

The findings of others, in so far as they are made known, are largely in agreement with ours. Thus, Moricard and Bize (21) in a boy, and Hamilton (24) in a eunuchoid, report increase in pubic hair; Moricard and Bize and Holt (25) in boys, and Baur and Koch (22) and Hamilton each in one eunuchoid report enlargement of the penis, whereas Hamilton alone seems to have as yet observed prostatic enlargement.

The influence of testosterone propionate on the testes themselves is still an open question. Careful measurements by calipers showed no change in the size of these organs in either *N. D.* or *N. T.*, despite practically daily treatment for 28 and 54 days respectively. In *N. T.*, however, a few sperm, consistently present before, disappeared during treatment to reappear after discontinuance. Certain doses, at least, of androgens (androsterone) have inhibited the developing seminiferous epithelium of growing rats, but have not materially affected adults (28). The matter is, however, quite complex, as testis extracts prolong the life of the sperm in the isolated epididymis of the castrated guinea-pig (29) and testosterone, together with other substances protect the seminifer-

ous epithelium of the rat from deterioration after hypophysectomy (30, 31, 32). McCullagh, McCullagh and Hicken (33) report an increase in the number of sperm cells, obtained after prostatic massage in certain men sexually deficient after treatment with urinary androgens. The influence on the seminiferous epithelium in man must be studied much further, but our experience with *N. T.* suggests that inhibition of sperm formation in already hypoplastic testes may be produced by testosterone propionate. Descent of the testes in monkeys (34) and in 2 eunuchoids has been produced by testosterone compounds (22, 24). We observed nothing of this sort in our 2 cryptorchids but previous surgery may well have interfered with the process.

The stimulation of breast tissue in *J. H.* by testosterone, on one occasion, and by testosterone propionate on another, is of interest. Hamilton has reported a similar finding in his eunuchoid, and several important observations of breast hypertrophy in animals after androgens have been reported (31, 35, 36). The common breast hypertrophy of the male adolescent (37) may thus be due to testosterone as such, although interconversion of the male hormone into some direct breast-stimulating substance is possible, in the light of the recent report of Steinach and Kun (38) indicating an increased excretion of estrogens after the administration of testosterone to man.

The consistent gain in weight, accompanied by fullness of the face and occasionally demonstrable edema, and occasionally by a marked increase in appetite is of the greatest interest, and is the subject of a special study to be reported subsequently (39). The precordial discomfort of *H. K.*, apparently produced by testosterone propionate, is the only deleterious action thus far known to us. Its mechanism is obscure, but it should be kept in mind. Otherwise our patients felt well, and their outlook was greatly improved by the evidences of male hormone activity.

SUMMARY

Four eunuchoids received 25 mg. daily of testosterone propionate in sesame oil, subcutaneously from 5 to 7 times weekly for 28 to 99 days. Thereafter, in 3 of these, 10 to 25 mg. was given from 3 to 7 times weekly, with interruptions, until the 108th to the 163rd days. There was in all of the patients an early increase in erections, in 3 an enlargement of the penis, in all an enlargement of the prostate, in 2 a distinct deepening of the voice, in 3 an increase in sexual hair. Hypertrophy of breast tissue occurred in 1 patient, the size of the testes was unaltered in the 2 studied, but sperm disappeared during treatment in 1 of these 2, to reappear later. In all 4 there was a marked increase in body weight, in 2 accompanied by increased appetite, and in 2 by evident edema. In 1 there was a slight increase in the B.M.R.

Addendum. The recent excellent paper of Vest and Howard (40) in which the effects of testosterone propionate in 6 hypogonad men and 2 prepubertal boys are detailed describes enlargement of the penis, scrotum, prostate and seminal vesicles, development of sexual hair, deepening of the voice and increased libido and potentia. They differ from the studies recorded here in finding slight enlargement of the testes. In one of their patients an increased number of sperm was found after treatment, together with motility not previously present, and this patient successfully impregnated his wife.

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THE EFFECT OF TESTOSTERONE PROPIONATE ON NITROGEN, ELECTROLYTE, WATER AND ENERGY METABOLISM IN EUNUCHOIDISM[1]

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In a preceding study of the effects of long-continued treatment of eunuchoids with testosterone propionate (1), a surprising and consistent increase in body weight was observed, amounting from 1.5 to 2.0 kg. in 10 days and to 4.5 to 8.5 kg. in 28 to 64 days. This increase in weight was, in all 4 patients, accompanied by fulness of the face, in 1 by pitting edema of the ankles, and in 1 by puffing of the hands, indicating a gain in body water. In 2, however, an unmistakable increase in food intake was reported by the patients, and although, in 3, reduction of the dose of testosterone propionate or its discontinuance led to a rapid loss of at least part of the weight gained, in 1 only (*H. K.*), an insignificant loss appeared, as if tissue had been materially augmented by the course of treatment. Studies in 1 patient (*N. T.*), through such a period of weight gain showed a slight rise in the B.M.R. indicating that depression of the resting oxygen consumption was not of importance. Moricard and Bize (2) in boys, and Foss (3) in a eunuch, had also noticed this gain in weight with testosterone compounds, as had Usui and his coworkers (4) with a urinary androgen in 3 eunuchoids. Korenchevsky, Dennison and Brovsin (5) report somewhat lower weights for the body as a whole, liver, heart and kidneys in castrated rats than in controls and found that certain dosages of testosterone elevated these weights toward normal. They speak accordingly of an anabolic property of testosterone.

While our work was in progress, Thorn and Harrop (6) published their discovery that retention of sodium, together with associated water, may be produced in the normal dog by the administration of estrone, estradiol, progresterone, pregnanediol and testosterone, an effect like that of adrenal cortical extracts in normal dogs (7), and in normal man (8, 9). Thorn and Harrop were able to simulate the 'sodium-retaining' action of adrenal cortical extracts in a patient with Addison's disease by estrone (6), and Thorn, Nelson and Thorn (10) have given physiological significance to these important observations by the suggestion that changes in weight of women during the menstrual cycle, together with menstrual edema, are due to such action of the female sex hormones. The chemical relationships between the adrenal and sex hor-

[1] A preliminary report of these observations has already appeared (16).

mones that are now becoming evident (11, 12) are thus seen to have biological corollaries.

Another important metabolic influence of the androgens, that of diminishing nitrogen excretion in the castrate male dog, has been recently established through the work of Kochakian and Murlin (13, 14) and Kochakian (15). In their experience with androsterone, androstene-dione, and testosterone, this decline in urinary nitrogen was accounted for by the change in the urea fraction, and was unaccompanied by any increase in the nitrogenous constituents in the blood. The protein stored, they felt, might be due to the growth of the prostate and seminal vesicles.

With these findings in mind we have analyzed, in part, the effects of a daily

TABLE 1. EFFECT OF TESTOSTERONE PROPIONATE (25 MG. DAILY) ON CERTAIN BLOOD CONSTITUENTS OF *N. D.*

Date	Plasma proteins gm./100 cc.	N.P.N. mg./100 cc.	Urea mg./100 cc.	Urea clear.	Hb. gm./100 cc.	Rbc. m./cu. mm.	Rbc. vol. %
9/22/37		26	13.3	52			
9/24/37	7.56		15.7	40			
9/29/37	6.38	34					
10/ 1/37			13.8	40			
10/ 5/37	6.84	31			12.7	4.510	42
10/ 6/37					13.0	4.510	42
10/ 9/37	6.74	20	12.1	73[1]			32(?)
10/11/37					13.0	4.600	
10/13/37	7.18	22	9.9	25	13.4	4.740	44
10/15/37	6.89	17	8.5	41			
10/16/37					13.4	4.600	44
10/19/37							42
10/21/37	5.88	20	11.4	40	13.4	4.630	42

[1] from formula $\frac{UV}{B}$. Other clearance values from $\frac{U}{B\sqrt{V}}$

Testosterone propionate given from 10/6/37 to and including 10/14/37. Although the testosterone propionate was given last on October 14th, the effect was still present on the 16th. Hemoglobin was determined by Newcomer, cell volume by hematocrit.

dose of 25 mg. of testosterone propionate[2] on 4 eunuchoids kept in the hospital on constant diets. Three of these men were alike in that the etiology of the condition was unknown, whereas the 4th had a suprasellar cyst, the hypogonadism, therefore, being of pituitary origin.

METHODS

The 4 patients were placed on weighed diets, constant in calories, carbohydrate, protein, fat, food water and minerals. The energy value was estimated so as to provide unchanging body weight, allowing for moderate, regulated activity during the day. This estimate was checked in patient *N. D.* by the measurements of insensible weight loss; in the others approximate checks were provided by the minimal variations in weight during the control periods. In 2, *F. R.* and *M. D.*, 3 alternating diets of similar composition, as judged by

[2] We are indebted to Drs. Erwin Schwenk, Gregory Stragnell, and Max Gilbert of the Schering Co. for generous supplies of testosterone propionate.

the standard food tables, were used; whereas in 2, *N. D.* and *H. S.*, the same food as nearly as possible was given each day. In *N. D.* the food was served hot and cold on alternate days, insensible weight loss being determined on the 'cold days.' Two gm. of added sodium chloride were given per day to each of the 4 patients. Two men (*F. R.* and *M. D.*) were available only in the

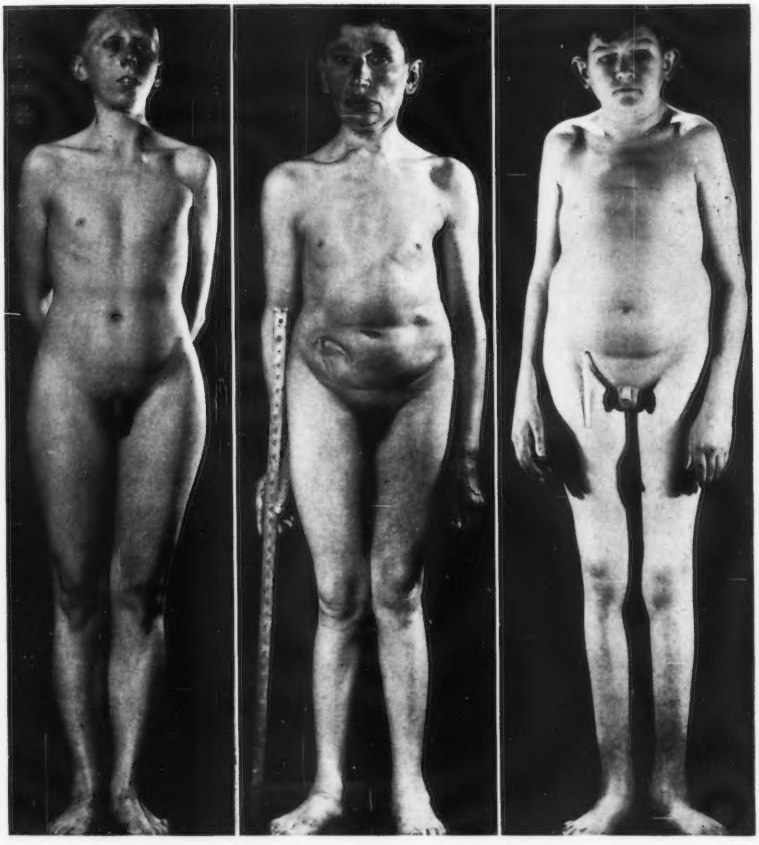

Fig. 1. Anterior views of *F. R.*, *M. D.* and *H. S.*, reading from left to right. For *N. D.* see preceding paper.

summer, and although sweating was minimized it must have occurred at times. The data on sodium and chloride are offered on these 2 only because they are in agreement with that in the others. *F. R.* and *M. D.* drank measured amounts of water as they chose, while *N. D.* and *H. S.* used fixed amounts.

The procedure for insensible weight loss was as follows. *N. D.* was weighed every morning on a chair balance which had a sensitivity of 5 gm. On alternate days the insensible loss of weight was determined in the usual way; all food, water, and excreta were weighed to the nearest gram. For each 24-hour period, the final body weight plus the weight of the excreta were

subtracted from the initial body weight plus the weight of the ingesta to obtain the insensible weight loss. Newburgh and his associates (17) have shown that the insensible loss of water, which accounts for about 90 per cent of the insensible loss of weight, is a function of the total metabolism. Thus, with care being taken to avoid visible perspiration, the measurement of the insensible weight loss serves indirectly as a gauge of the total activity of *N. D.* during the observation.

The urine for each day was kept in the ice-box at all times, and at the end of each 24 hours an aliquot was removed for chemical analysis and the remainder set aside for assay for sex hormones. In *F. R.* the urine was acid; in the first *M. D.* experiment it was neutral to alkaline, in the second *M. D.* experiment and in *N. D.* and *H. S.* acidity was insured by the addition of 10 cc. of 5 N sulphuric acid to the container. Except where otherwise noted, chemical determinations were made in the clinical chemistry laboratory of the Department of Medicine by procedures which Peters and Van Slyke (18) give in detail. Sodium in urine, Barber and Kolthoff; potassium in urine, Clausen, Kramer and Tisdall; chloride in urine, Volhard and Henry; creatine and creatinine in urine, Folin (creatine by autoclave); urea N in blood and in urine by the gasometric urease method of Van Slyke; total N in blood and urine, macro Kjeldahl (19); plasma proteins, Campbell and Hanna (20).

Basal metabolic rate determinations in all were made by the Benedict-Roth and in *F. R.* and *M. D.* by the gasometer methods (21), and the Mayo Foundation standards (22) were used for comparison.

PROTOCOLS

F. R., aged 27 years (University of Chicago Clinics No. 113589) was referred by Doctors Carl Moore and C. B. Huggins. The eunuchoidism (fig. 1) was of uncertain origin, although a serious head injury and an infection or dermatitis involving the legs and scrotum in childhood presented possibilities of injury both to the pituitary body and to the testes. His standing height was 177.1 cm., sitting height 89.3 cm., sitting:standing ratio 50.4, greatest arm stretch 192 cm., ratio span:stature 108.4. His weight was 73.7 kg. and there was a considerable deposit of fat about the hips, giving a feminine contour to this region. Pubic, axillary and facial hair was scant, and his smooth face required no shaving. His voice was a rather high-pitched masculine one. The penis was from 5.3 to 6.4 cm. in length and 2.6 cm. wide at the corona. The testes measured 2.7 x 1.8 x 1.5 cm. Small prostatic lobes estimated as 4 mm. wide and 6 mm. long could be felt. Visual fields and x-rays of the sella turcica showed no abnormalities. X-rays on April 12, 1937 showed persistence of the line of epiphysial union at the head of the radius, from non-union to partial union of epiphyses with their corresponding metaphyses at the lower ends of the radius and ulna, bases of metacarpal I and proximal two rows of phalanges and heads of metacarpals II to V. Epiphyses about the knees were still incompletely fused. Those at the hip were fused. The delay may be estimated as about 6 years. A glucose tolerance after 72 gm. of glucose orally gave 65 mg./100 cc. fasting, 116 at ½ hr., 33 at 2 hr. and 88 at 3 hr. He had a mild hypochromic anemia with a r.b.c. of 4,100,000, Hb. of 80 per cent (oxygen capacity = 16.5 vol. per cent), hematocrit showing 33 per cent cells. His urine was normal.

On May 27, 1937, the patient entered the surgical service of Dr. H. P. Jenkins,

and an appendectomy was performed for acute appendicitis. His convalescence was satisfactory. On June 9th he was placed on the dietary regime of C 204-211, P 75-78, F 108-116, Calories 2106-2196, after 4 preliminary days, in which the carbohydrate

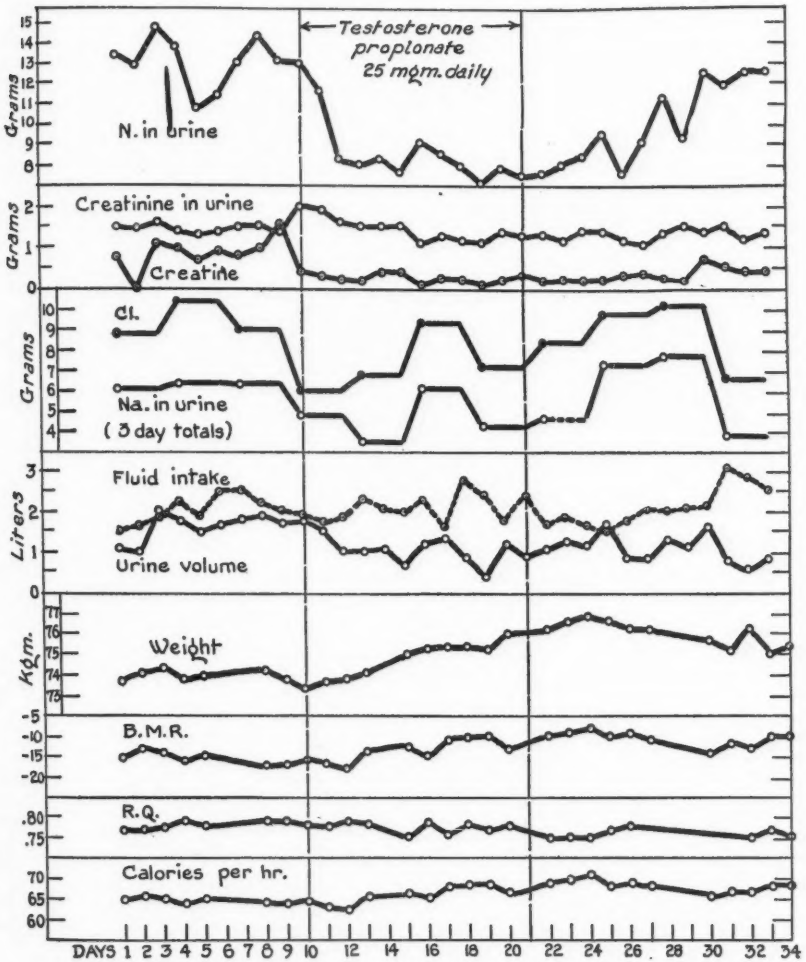

Fig. 2. The effect of testosterone propionate on *F. R.* This experiment extended from June 7th to and including July 10, 1937. Testosterone propionate was given from the 10th to and including the 21st day. The drop in urinary sodium and chloride and in urinary volume and increase in water intake, beginning on day 31, occurred during very hot weather. The dotted line for sodium at day 22 signifies that 2 analyses only were made for this 3-day period.

varied from 202 to 230 gm. per day, the other constituents remaining the same. Three diets of similar composition were alternated. As estimated from food tables he received daily from 2.4 to 2.9 gm. of chloride, from 1.3 to 1.9 gm. of sodium, from 3.0 to 4.1 gm. of potassium and from 1290 to 1390 gm. of water in his food. In addition, 2 gm. of sodium chloride were given as such. His water consumption

was unregulated. Certain changes to meet the taste of the patient and to provide him meat-less Fridays were necessary. On June 11th, he received 85.5 gm. of protein and 3.9 gm. of food chloride, on June 18th, 69.6 gm. of protein, on June 25th, 2.03 gm. of food sodium. On June 30th and July 3rd, the fat was increased to 130 gm., giving a caloric value of 2300 to the diet for those days. Two errors were made, for which allowances are possible. On June 9th he took only 1.4 gm. of the added salt instead of the standard 2.0 gm., and on June 16th 2.0 gm. of sodium bicarbonate were given by a house officer unfamiliar with the problem.

The patient was allowed to be up and about the hospital, and took a walk each afternoon. His weight was maintained between 73.4 and 74.3 kg. during the 9 days before testosterone, but he averaged a negative nitrogen balance of 0.59 gm. per day (estimated), omitting fecal nitrogen. Sweating was avoided when possible, but doubtless occurred. It was unfortunate that this patient had to be studied in the summer, but it was either then or not at all.

Between June 16th and 27th he received 12 intramuscular injections, of 25 mg. each, of testosterone propionate in sesame oil. The metabolic effects are shown in figure 2. By June 19th (day 13, fig. 2) a slight increase in the frequency of erections appeared, and by the 23rd (day 17) they became nearly constant. On day 18 the scrotum was sufficiently sore to cause discomfort on walking, and was markedly edematous. On the 26th (day 20) the prostate was definitely larger, each lobe being estimated at 1.0-1.5 cm. broad. The penis was 6.8 cm. long on the 28th (day 22) and 8.2 cm. long on the 30th (day 24). Eight days after the last injection the erections were recorded as subsiding gradually, the penis had returned to a length of 6.5 cm. and the prostate was still enlarged but questionably softer. July 10th, 12 days after the last injection, matters were much the same. The testes as measured by calipers at no time changed in size. It is of interest that this patient at no time admitted any improved sense of well-being, complaining not infrequently of slight dizziness and nausea, and that he was disconcerted and displeased by the sexual stimulation. When he returned to our care on September 29th after a vacation, his prostate had returned to the original size.

M. D., aged 51 years (University of Chicago Clinics No. 176188), was referred by Dr. S. J. Glass of Los Angeles, Calif. This patient (fig. 1) was seen many years ago by Falta in Vienna, and is shown at the age of 20 in Falta's well-known textbook (23). His standing height was 157.5 cm., sitting height 79.3 cm., sitting:standing ratio 50.3, greatest arm stretch 172.8 cm., ratio span:stature 109.7. Hrdlička's tables give a sitting:standing ratio of 52.76 for Jews in America and span:stature ratios of 103.3-103.8 for various groups of European Jews (24). These values illustrate the eunuchoid disproportion. This patient weighed 50 kg., but there was, despite the general emaciation, a definite pad of supra-pubic fat. Genu valgum was present. His skin was soft and atrophic and thrown into many fine wrinkles about the face. Pubic, axillary, limb and facial hair was scant. His voice was of fairly normal pitch but not deep, and was somewhat husky. The penis was 2.8 cm. long and 1.3 cm. wide at the corona. The testes were 2.1 x 1.3 x 1.2 cm. A small flat prostate could be felt. Visual fields were normal, but there was an enlargement of the blind spot in the left eye. The sella turcica was normal, and the epiphyses at the shoulder, wrist and hand, hip and knee were closed. There was considerable osteoporosis. Glucose tolerance was normal. The Wassermann and Kahn tests on the blood serum were negative, r.b.c. 4,000,000/cu. mm., Hb. 79 per cent (Sahli), w.b.c. 72,000, 53 per cent polymorphonuclears, 36 per cent large lymphocytes, 8 per cent small lymphocytes, 3 per cent mononuclears.

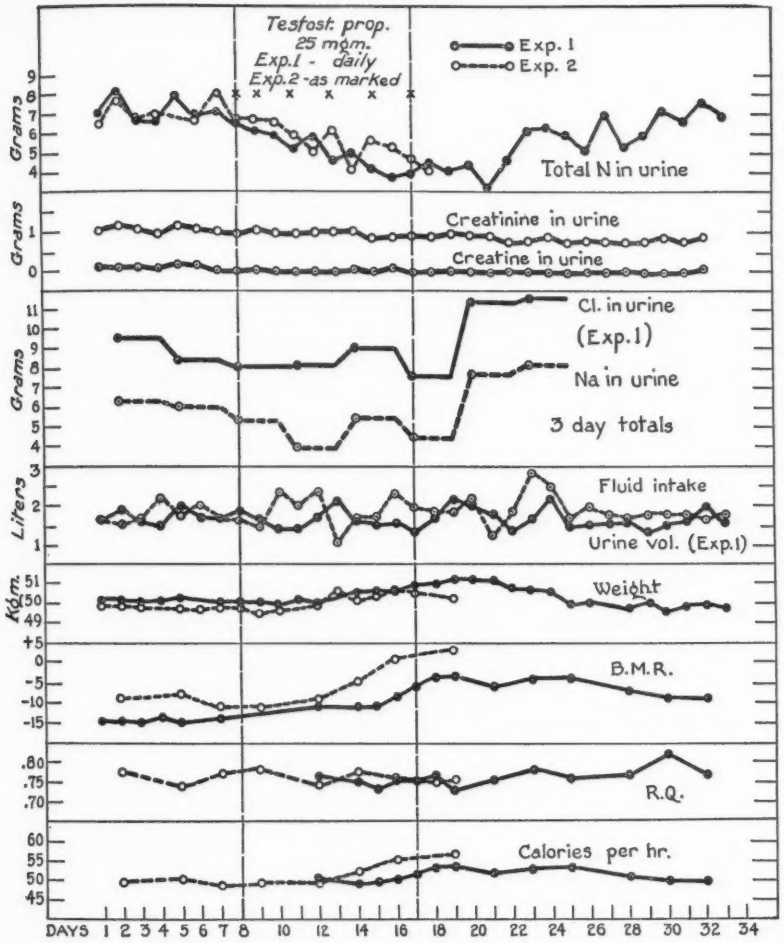

Fig. 3. The effect of testosterone propionate on *M. D.* The first experiment extended from July 13th to August 14, 1937, the second from August 12th to and including August 30th. The B.M.R. determinations were made by Benedict-Roth from day 1 to 7 of the first experiment, and thereafter, by the gasometer. Creatinine, creatine, chloride, sodium in the urine and fluid intake and urine volume are given for Exp. *1* only.

The patient was extremely nervous and anxious, and suffered greatly from a sense of inferiority. He had many visceral complaints, especially an epigastric burning and frequency and discomfort on urination. A bowel resection had been performed 9 years previously for tuberculosis of the colon. Dr. Glass had, in May 1936, demonstrated a diverticulum of the bladder by cystoscopic and urographic means. The mucosa of the bladder appeared normal except for inflammation at the posterior lip of the neck of the bladder. Urographic examination of the kidneys by skiodan intravenously showed no lesions. The examinations of Dr. Glass and our own repeated studies showed no abnormalities of the urinary sediment. X-ray studies of the

chest, gall-bladder, stomach, duodenum, and colon were normal at the University of Chicago, and many stool examinations were negative for blood. Nevertheless, the patient ran a slight fever (99°-100°) much of the time, and this together with the sedimentation rate of 30 mm. in one hour suggested a low grade infection, the site of which was never ascertained.

The patient entered the hospital on June 20, 1937. On July 7, 1937, he was placed on the dietary regime of C 190-204, P 62-64, F 76-79, Calories 1718-1781. Three diets of similar composition were alternated. As estimated from food tables, he received daily from 1.93 to 2.13 gm. of chloride, from 1.81 to 1.93 gm. of sodium, from 3.32 to 3.53 gm. of potassium, in addition to an unknown but constant amount from the 30 gm. of 'Vitavose,' which he took daily. Food water varied from 1211 to 1267 gm. daily. Two gm. of sodium chloride were given daily as such. His water consumption was unregulated. One gm. of sodium bicarbonate was inadvertently given on July 18th and is allowed for in the excretion studies submitted. He received 30 minims of *Tr. Belladonna* daily, and between August 16th and 29th, 30 mg. of phenobarbital daily.

He was allowed to be up and about, and took a walk each afternoon. His weight was maintained between 50.0 and 50.3 kg. during the 7-day control period. Collections of urine were started on July 13th, and during the 7 control days he was estimated to be in positive nitrogen balance of 2.73 gm. per day, omitting fecal nitrogen. Sweating was avoided as much as possible. In this case, as in *F. R.*, the patient was available to us only in the summer.

Between July 20th and July 29th he received 10 daily injections of 25 mg. each of testosterone propionate in sesame oil. The metabolic effects are shown in figure 3. The evening of July 22nd (day 10, fig. 3) a great increase in the frequency of erections was noted. By day 16 the erections were constant, the priapism keeping the patient awake much of the night. It was not possible to measure the penis while it was at rest. By day 16 the prostate was not larger in area, but was much fuller than previously. This entire process excited the patient greatly, and the changes in B.M.R. recorded are perhaps questionable. Six days after stopping the testosterone propionate the erections began subsiding, and they had completely subsided by 8 days after. The psychic element here appeared large, and he was accordingly given 1 cc. of sesame oil every other day between August 10th and 18th, with the result that erections were again greatly stimulated.

On August 19th testosterone propionate, 25 mg. per dose, was given on alternate days to and including August 30th, 6 injections in all. On September 1st the observations were closed. The patient reported a great increase in erections during the last course of treatment, but no change in the prostate was observed.

N. D., eunuchoid, aged 31 years (University of Chicago Clinics No. 152001), was referred by Dr. M. Kolovros of Gary, Ind. (fig. 1). The record of the examination is given in the preceding paper. On September 19, 1937, he entered the hospital much the same as before his previous course of testosterone propionate. His penis was 7.2 cm. long and 2.8 cm. wide at the corona. The testes measured 2.5 x 1.6 x 1.4 cm. The prostate was very small and flat.

After 8 days on preliminary diets of approximately the same composition in carbohydrate, protein and fat, he was placed on a constant experimental diet of C 188, P 60, F 103, calories 1919, food water 1403. The same foodstuffs were used throughout, save that peas and asparagus were alternated, and mayonnaise substituted for part of the butter every other day. The vegetables and fruit were from the same case of canned goods, and 2 canned hams served as the meat, the second being

started on October 13th. The meals were prepared hot and cold on alternate days, the cold meals being used to permit accurate weighings for the determination of insensible weight loss, as described under the heading Methods. The butter was

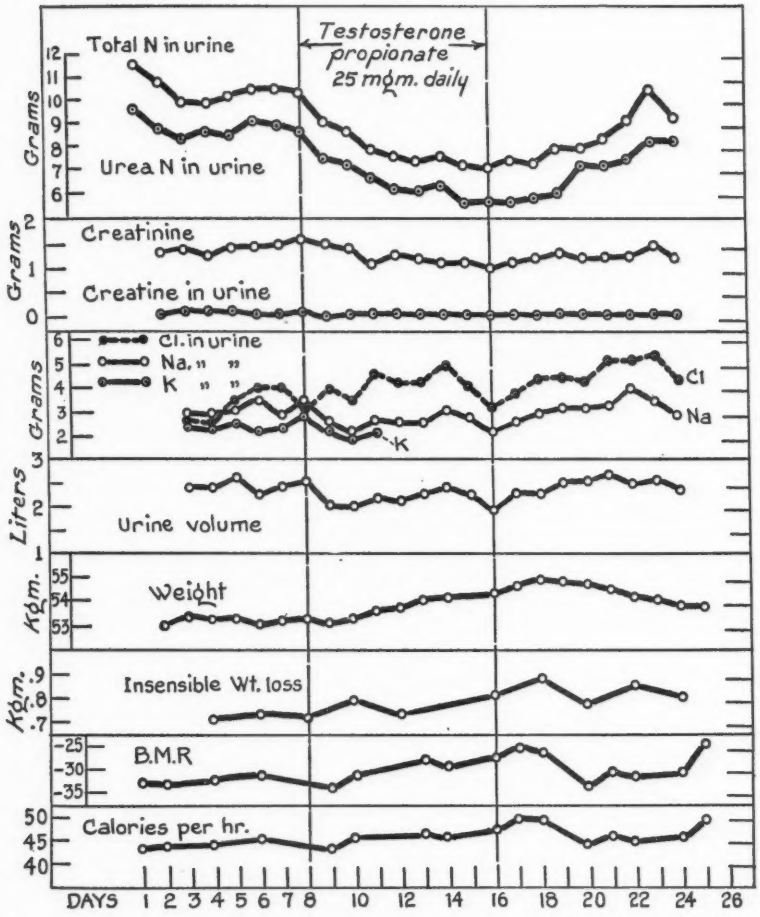

Fig. 4. The effect of testosterone propionate on *N. D.* extending from September 29th, to and including October 23, 1937. The fluid intake was constant, and included 1500 cc. of water daily.

salt-free, except on October 19th and 20th, when salted butter was served through an error. Analyses of the chloride content of the butter used permitted corrections in the mineral excretion on these days. The sodium content of the diet was 0.47 gm., chloride 0.89 gm., potassium 2.7 gm. in addition to that provided by the ham on which figures were not available. Two gm. of sodium chloride was provided daily as such. He drank 1500 cc. of distilled water daily, and the dishes used were washed in distilled water.

During the experiment the patient was allowed a fairly constant amount of ac-

tivity about the hospital and in daily walks outside. During the 7-day control period, from September 29th to and including October 5th, he maintained his weight between 53 and 53.3 kg., and was in an estimated negative nitrogen balance of 0.8 gm. daily, not counting fecal nitrogen. Between October 6th and 14th he received 9 daily intramuscular injections of 25 mg. each of testosterone propionate in sesame oil. On October 11th (day 13, fig. 4) erections were reported as increased, but the patient never emphasized this change and seemed reluctant to discuss the matter. The penis elongated slightly to 7.9 cm. on day 17, and resumed the original length of 7.1 cm., 6 days after stopping treatment. By day 14 (October 12th) the prostate had doubled in size, and 5 days after stopping the injections it had again reached the original size. The after-period lasted for 8 days until the morning of October 23rd. The metabolic effects are presented in figure 4 and in table 1.

H. S. eunuchoid, aged 24 (University of Chicago Clinics No. 186519) was referred by Dr. I. Becker of the University of Illinois (fig. 1). This patient was 171 cm. tall; his sitting height 77.1 cm., ratio of sitting: standing heights 45.1, the lowest in our series of 8 eunuchoids. Several skeletal deformities were present, including a severe pes planus of the left foot, limitation of extension of the forearms, due apparently to distorted growth of the olecranon, and limitation in abduction of the arms, not fully accounted for. The chest was narrow above, and flared toward the base. The epiphyses of the phalanges, metacarpals and distal ends of the radius and ulna were unfused, as was that of the head of the humerus, while that of the clavicle was closed. At the elbow the humeral epiphysis was closed, whereas those of the radius and ulna were still open peripherally. The pelvic epiphyses were not apparent except those of the Y-cartilages of the acetabulum which were not closed. The femoral neck was unusually long, the greater trochanter large, and the capital and trochanteric epiphyses were unfused. The patella was large and irregular, and the epiphyses about the knee were unfused except that of the tibial tubercle. The distal epiphyses of the tibia and fibula were unfused, the apophysis of the calcaneus had united to the calcaneus, the great toe was unusually long, the naviculars flattened and elongated, the epiphyses of the metacarpals were ununited. The delay in epiphyseal closure may here be estimated at fully 6 years.[3] His weight was 64.3 kg., a suprapubic fat pad was present, and the abdomen was full and protuberant. There was no terminal pubic, axillary, limb or facial hair, the skin was smooth and boyish. His penis was 3.9 cm. long and 1.7 cm. wide at the corona. The scrotum was small and the testes 2.0 by 1.3 by 1.3 cm. The prostate could not be felt. Erections were rarely present, although the patient's story was variable in this regard. His voice was boyish, and the laryngeal prominence could not be felt.

X-ray of the skull showed an enormous dilatation of the sella turcica, with an antero-posterior diameter of 35 mm. and a depth of 15 mm. About 45 mm. above the sella were scattered patches of amorphous calcification, the area thus involved measuring 4 cm. in diameter. The optic discs were those of primary optic atrophy. There was no light perception in the left eye, and in the right eye vision was present only in the nasal field.

The Wassermann and Kahn tests on the serum were negative, r.b.c.: 4,470,000, Hb. 12.4 gm. (Newcomer), hematocrit 39 per cent, giving a mean corpuscular volume of 87.2 cu. micra and a mean corpuscular hemoglobin of 27.7, wbc. 7,500 with 61 per cent polymorphonuclears. 33 per cent lymphocytes, 4 per cent mononuclears and 2 per cent eosinophiles. A glucose tolerance test was normal.

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[3] We are indebted to Dr. C. H. Hatcher for his assistance in the analysis of the skeletal deformities.

On December 3, 1937, he was placed on a constant diet of C 197, P 64, F 100, Calories 1942, food water 1313. Vegetables and fruits from the same case were used and 3 sirloin butts, started respectively on December 3rd, December 19th and January 10th served as the meat. Salt-free butter was given throughout. As estimated from food tables, this diet contained 0.6 gm. of sodium, 0.9 gm. of chloride, and 2.7 gm. of potassium per day. Two gm. of sodium chloride was given daily as such. He

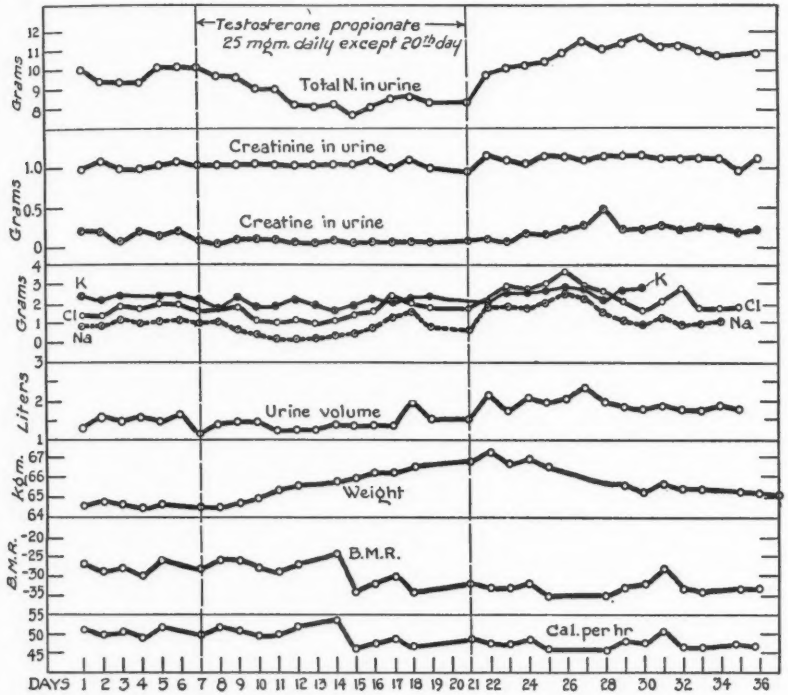

Fig. 5. The effect of testosterone propionate on *H. S.*, who had a suprasellar cyst. The observations extend from December 7, 1937 to and including January 12, 1938. The fluid intake was constant, and included 1200 cc. of water daily.

drank 1000 cc. of distilled water on December 5th and 6th, and thereafter 1200 cc. daily. The dishes were washed in distilled water.

He was allowed to be up and about the hospital and, on occasion as weather permitted, walked outside. His weight was maintained between 64.4 and 64.8 kg. during the 8 control days, and he was in an estimated positive nitrogen balance of 0.4 gm. daily, not counting fecal nitrogen.

From December 13th to December 27th he received daily, 25 mg. of testosterone propionate intramuscularly, with the exception of December 26th, making 14 injections in all. Increased erections were noted by day 21 (fig. 5), but the patient volunteered nothing concerning these, and his testimony gave every evidence of uncertainty. The prostate by this time was, however, clearly definable as a thin sheet of tissue the area of a quarter. There was some edema of the scrotum, and the penis was 5.7 cm. long and 2.2 cm. wide, substantially larger than before. The testes were

TABLE 2. THE EFFECT OF TESTOSTERONE PROPIONATE ON THE URINARY EXCRETION OF NITROGEN AND ELECTROLYTES

	F. R.			*M. D.* (1)			*M. D.* (2)			*N. D.*			*H. S.*		
	Control	Exper.	Recov.	Control	Exper.	Recov.	Control	Exper.	Recov.	Control	Exper.	Recov.	Control	Exper.	Recov.
	9 da.	14 da.	7 da.	7 da.	11 da.	7 da.	7 da.	9 da.		5 da.	10 da.	7 da.	6 da.	15 da.	10 da.
Average N Intake/day	12.46			10.01			10.01			9.60			10.2		
Average N in urine/day	13.05	8.54	9.53	7.26	5.14	5.07	7.28	5.89		10.39	8.00	8.58	9.8	8.64	10.74
Estimated N balance/day[1]	−.59[1]	+4.51	3.52	+2.75[1]	+2.12	+2.19	+2.73[1]	+1.39		−.79[1]	+2.39	+1.81	+.4[1]	+1.16	−.94
Total N balance		+63.14	+24.6		+23.3	+13.69		+12.51			+23.9	+12.67		+17.4	−9.40
Total protein balance		+394.63	+153.8		+145.6	+54.76		+78.19			+149.4	+79.18		+108.8	−58.8
Av. Na in urine gm./day	2.09	1.54	2.51	2.02	1.64	2.51				3.04	2.69	3.29	1.06	.73	1.78
Estimated Na balance gm./day		+.55	−.42		+.38	−.49					+.35	−.25		+.33	−.72
Total Na balance gm.		+7.70	−2.94		+4.18	−3.43					+3.50	−1.75		+4.95	−7.20
Total Na balance (m. eq.)		+335	−128		+184	−149					+152	−76		+215	−313
Av. Cl in urine gm. per day	3.15	2.48	3.40	3.16	2.90	3.64				3.60	4.01	4.76	1.78	1.58	2.66
Estimated Cl balance gm./day		+.67	−.25		+.26	−.48					−.41	−1.16		+.20	−.88
Total Cl balance gm.		+9.38	−1.75		+2.86	−3.36					−4.10	−5.25		+3.00	−8.80
Total Cl balance (m. eq.)		+264	−50		+80	−95					−113	−148		+85	−248
Av. K in urine gm. per day										2.60	2.25 (4 days)		2.36	2.09	2.55 (7 days)
Estimated K balance gm./day											+.35			+.27	−.19
Total K balance gm.														+4.05	−1.90
Total K balance (m. eq.)														+104	−49
Change in body weight in grams		+3500	−1700		+1300	−1200		+900			+1670	−1001		+2800	−1800
Change in body wt. from protein×4		+1579	+598		+582	+219		+313			+598	+317		+435	−235

[1] The estimated N balance during the control period is derived from the N in the urine and from the protein content of foods given in standard tables. Otherwise the estimated balances are obtained by comparison with the average excretion during the control periods. They are estimations only, and are not to be treated as exact values. The 'experimental' columns cover the period from the beginning of testosterone propionate to the peak of weight gain. The 'recovery' columns cover periods for which data for the several constituents of urine are complete, and not the entire series of observations in all cases. The weight gained from protein is calculated on the assumption that three grams of water are stored with each gram of protein.

unaltered. Enuresis appeared the night of December 26th, spoiling this specimen, and some loss of urine with defecation occurred on December 27th. He was thereafter wakened repeatedly during the night and further significant losses prevented. Areas of wetting, 5 cm. in diameter, were present on his pajamas on January 1st and 4th, but inspection of the figure given presents no evidence that these losses were anything more than they appeared to be.

Following the completion of our studies, this patient was returned to the University of Illinois Hospital. Dr. Eric Oldberg attempted to deal surgically with the enormous cyst, but could remove only a few cubic centimeters of fluid, and the patient succumbed the second day thereafter. At autopsy an "enormous tumor was found which had destroyed the pituitary body and had tremendously distorted the optic chiasm and the circle of Willis."

DISCUSSION

In the eunuchoid, testosterone propionate consistently produces a striking drop in the urinary total nitrogen (fig. 2, 3, 4, 5), which is reflected completely in the urinary urea (fig. 4), and which is unaccompanied by any regular change in the urinary creatinine or by increase in the urea, non-protein nitrogen, plasma proteins or hemoglobin of the blood (table 1). The urinary nitrogen began to decline from the 1st to the 3rd day after starting testosterone propionate, reached minimum values from the 6th to the 13th days, and began increasing from the 1st to the 6th days after the last injection, approximating or exceeding the baseline from the 1st to the 10th days after the last injection. This effect is in entire agreement with the results of Kochakian and Murlin (13, 14) with urinary androgens and synthetic androstene-dione, and of Kochakian (15) with testosterone and testosterone acetate in the castrate dog. Indeed, the estimated nitrogen stored in our experiments, 0.02 to 0.06 gm. per kilo per day (table 2), precisely corresponds to Kochakian's for testosterone acetate. During recovery, the heightened nitrogen excretion exceeded the baseline only in *H. S.* (fig. 5), who lost all of his previous gain. *M. D.* (fig. 3) was observed for 21 days after the last injection of testosterone propionate without any signs of loss of the stored nitrogen. The Rochester workers, usually, but not always, observed a hypernormal excretion during recovery, but noted that part of the nitrogen gained could be tenaciously held for long periods.

The repository of the nitrogen stored in the body under the influence of the androgens remains uncertain. From 108.8 (*H. S.*) to 394.6 gm. (*F. R.*) of protein may be estimated as retained. The normal young adult prostate weighs 20 gm., and although it was clear enough, from our examinations that the prostate increased in size, it was equally clear that it never attained normal dimensions, and the same may be assumed for the seminal vesicles, which follow the prostate in normal development. Only a small fraction, not more than 10 gm., of the protein could go to form new tissue in the accessories. Some 5 gm. of nitrogen may be retained in the augmented extracellular fluid of the body (*N. D.*). The fate of the remainder is speculative. Korenchevsky, Dennison and Brovsin (5) have reported that testosterone increases the size of the somewhat smaller than normal livers, kidneys and hearts of castrated

rats, so that some new protein may well go to these organs. But consideration of such facts as the extraordinary muscular development in certain instances of precocious puberty suggests that muscle must also be considered as a possible site of new tissue formation.[4]

Fat may substitute in the metabolic mixture for the protein spared, as concluded by Kochakian and Murlin for the dog. Calculations in *N. D.* (fig. 6) from the total calories expended, urinary nitrogen, and carbohydrate intake (Newburgh, et al [17]) show a slight increase in fat consumption as the urinary nitrogen declines, as is inevitable from the assumption made that carbohydrate utilization equals carbohydrate ingestion. The fasting R. Q.'s given for *F. R.* and *M. D.* show little change. From calculations of the non-protein respiration quotient (after Lusk [25]) of *F. R.*, there is a slight increase in fat consumption as the nitrogen excretion declines, but such data is fragmentary from the standpoint of the metabolic mixture of the entire day, and unsatisfactory because of the nature of the respiratory quotient as a resultant of numerous opposing processes. Studies of nitrogen excretion after castration are entirely too fragmentary to permit comparison with the nitrogen-sparing effect of testosterone propionate. Korenchevsky (26) reported reduction of urinary nitrogen in the dog after castration, but his investigations were made some time after the operation. Kochakian and Murlin (14) could not obtain the nitrogen-sparing action of androstene-dione in the normal dog, a finding which indicates that this reaction is a genuine replacement effect.

We have obtained some evidence that creatinuria, when considerable in the eunuchoid living on a mixed diet, may be diminished by testosterone propionate. Thus, *F. R.* (fig. 2) averaged 844 mg. per day before treatment, 248 mg. per day during treatment, and showed a rise to between 420 and 670 mg. during the last 4 days of the recovery period. In *M. D.* (fig. 3), and *N. D.* (fig. 4), the spontaneous creatinuria was so irregular and slight (20 to 160 mg. per day) that it is difficult to feel sure of any influence of the androgen. In *H. S.* (fig. 5), the creatinuria was constant, and the drop from an average daily excretion of 165 mg. to an average of 65 mg. with a rise during recovery to 208 mg. is significant. As all of our patients except *M. D.*, who ate no meat, received considerable creatine in their diet, we can provide no information concerning the relative behavior of exogenous and endogenous creatine. The considerable literature which has grown up about the influence of the gonads on creatine metabolism cannot be reviewed here. Koch (27) has discussed some of this material, and pointed out the need for further careful study by better methods. The studies reported here, however, support the views of Bühler (28), Schittenhelm and Bühler (29) and others, to the extent that the androgens do modify creatine metabolism, and it is therefore entirely reasonable that their physiological secretion plays some part, at least, in diminishing creatinuria with the advent of adolescence, and that their absence is responsible for the occasional striking creatinuria of the eunuchoid.

[4] Since this paper was written an important communication by Papanicolaou and Falk has appeared (35) in which it was observed that the temporal muscles of the male guinea pig are larger than those of the female, that castration reduces their size and that testosterone propionate causes hypertrophy of these muscles in the castrated immature male and in the spayed and normal adult female. The general muscle mass is also similarly affected. It is thus highly probable that some of the nitrogen stored in our eunuchoids went to the muscles.

The estimated retention of sodium (0.33 to 0.55 gm. per day), and of chloride (0.20 to 0.67 gm. per day) in all but *N. D.* (table 2, fig. 2, 3, 4, 5) produced by testosterone propionate is in agreement with the findings of Thorn and Harrop (6) that this androgen possesses a 'sodium-retaining' effect in the normal dog in common with its relatives, estrone, estradiol, pregnanediol, progesterone and the adrenal cortical hormone. The decline in urinary sodium was fairly well marked by the 3rd day, reached values of as low as a third of normal (*H. S.*) within the first 6 days, and was fairly well

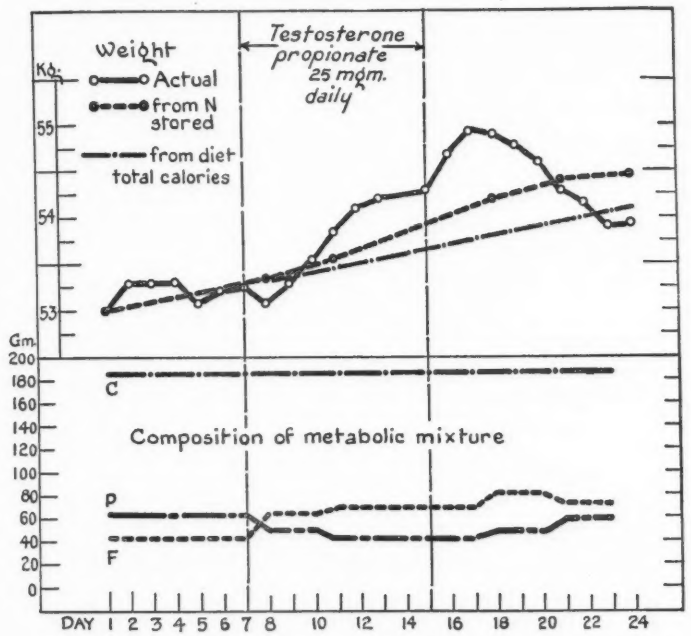

Fig. 6. Comparison of the actual weight gain in *N. D.*, with that expected from the nitrogen stored and with that predicted from the excess of diet calories over total calories expended. The curve of weight gain from nitrogen stored is calculated with the assumption that 3 gm. of water is deposited with each gram of protein stored. The total calories expended were derived from the insensible loss of water [calculated from the insensible weight loss, urinary N and carbohydrate of the diet after Newburgh et al (17)], and the third curve represents the gain in body weight which might be expected from the slight disproportion between diet calories and total calories, had not testosterone been given, and had the conditions of the control period been maintained throughout the observation.

The lower half of the figure illustrates the shift in metabolic mixture resulting from the administration of testosterone. The assumption is made that carbohydrate oxidized equals carbohydrate ingested.

sustained, although certain interruptions occurred. During recovery, the excretion of both sodium and chloride was sharply increased, and a hypernormal phase ensued, appearing from 1 to 6 days after the last injection. The rebound, which was more fully studied in *H. S.*, was of 10 days' duration, and resulted in the loss of slightly more sodium and considerably more chloride from the body than had been retained during the testosterone effect; estimations of 7.17 gm. of sodium and 8.79 gm. of chloride lost, as compared to 4.95 and 3.00 gained, respectively. It should be noted that the summer-time experiments

on *F. R.* and *M. D.*, which are only corroborative, show the same pattern and approximately the same quantitative results during the testoserone effect as the more satisfactory observations on *N. D.* and *H. S.* (table 2). A reduction in urine volume occurred with treatment, and a diuresis during recovery. Following the conceptions of Loeb (30) and Harrop (31) and their co-workers, concerning the mechanism of action of the adrenal cortical substance on electrolytes, it is probable that testosterone propionate likewise acts upon the kidney, but we can bring forward no direct evidence on this point. The failure to find increased potassium excretion (*N. D.*, *H. S.*) is of interest, as this commonly accompanies the 'sodium-retaining' action of the adrenal cortical hormone (7). Should this prove consistent, it may mean retention of potassium with nitrogen in new tissue, or the failure of testosterone to possess adrenomimetic properties in this regard. The possible physiological significance of these findings is completely unexplored. Inasmuch as Thorn and his co-workers (10) have found that changes in weight and in sodium excretion during the normal menstrual cycle may well depend upon the fluctuating secretion of the estrogens and progesterone, it would be unwise as yet to dismiss our observations as of pharmacological interest only.

The slight elevation of the B.M.R. in all instances except *H. S.*, who was hypopituitary, paralleling as it does the other more distinct reactions to testosterone propionate, is too consistent to be disregarded, although it is quite possible that subjective excitement rather than an immediate calorigenic influence is at work. This psychological influence seems especially likely in *M. D.*, who was greatly aroused by the sexual stimulation, and slept poorly. Our series of 8 eunuchoids (not including *H. S.*) showed an average initial B.M.R. of —11 per cent (range 0 to —19) by the Mayo Foundation Standards (21), whereas repetition to secure baselines for experimental purposes gave in 7 of these an average of —21 (range —14 to —32). As our only standards are based upon initial satisfactory tests, we must conclude that the eunuchoid has on the average a fairly normal basal rate, with a tendency, however, toward low values on occasion. McCullagh and Renshaw (32) in their series of 9 eunuchs, similarly record a range of +5 to —21 with an average of —13. The slight rise, therefore, of 6 to 14 points after testosterone propionate is about the most influence that one could expect the testis to have on basal oxygen consumption. The careful studies of Kochakian and Murlin (13, 14, 15) produced evidence of such an increase in basal metabolism in only one of several animals studied, a fat castrate dog treated with urinary androgens. Two of our 4 patients, including *N. T.* (1), reacting in this way were thin, and 2 were of normal weight. Whether the differences between the Rochester group and ourselves lie in the greater excitability of man, or elsewhere, must be decided after longer experience with the problem.

Testosterone propionate produces a uniform gain in body weight of from 1.0 to 2.6 kg. per 10-day period when given in 25 mg. doses daily, with a subsequent decline during recovery. The magnitude of this change, obtained in those on a constant hospital diet, conforms to that secured in the out-patient department (1), in which food and activity were unrestricted (*J. H.*, 1.5 kg.;

H. K., 1.5 kg.; *N. T.*, 1.5 kg.; *N. D.*, 2.0 kg. per 10-day period). It is probable from this out-patient experience that, had hospital treatment been protracted, plateaus in the curves of weight gain would have been reached between the 40th and 65th days of treatment. The meaning of this phenomenon has been clarified to some extent. However much a factor increased appetite (*J. H.*, *H. K.*) may be in long experiments (1) when the food intake is unlimited, it is not necessary for the weight gain, nor is depression of the basal oxygen consumption important. Observations on *N. D.*, by the insensible weight loss technic, provide no evidence of a decline in total caloric expenditure. The retention of 17.4 to 63.1 gm. of nitrogen, built into 108.8 to 394.6 gm. of protein, and assumed to store 3 gm. of water for each gram of protein,[5] provides for a gain in body weight of 435 to 1579 gm. (table 2). This would account for 1/7 (*H. S.*) to ½ (*F. R.*) of the total. The relation between the actual gain and the theoretical gain from protein stored, throughout the experiment on *N. D.*, is shown in figure 6. The fullness of the face, and the occasional obvious edema in the long experiments speaks for retention of extracellular fluid, and the decline in the urinary sodium and chloride supports this. Inasmuch as our experiments were designed to illustrate the general course of events and not to present exact electrolyte and water balances, we will not pretend to relate the mineral and water gains in a strictly quantitative fashion. Assuming, however, a concentration of 150 millimols of sodium per liter of retained water (34) for purposes of discussion, the 3.5 to 7.7 gm. of sodium estimated to be retained in our experiments provides associated water of from 1.0 to 2.2 kg., enough or more than enough to make up the difference between actual gain of body weight and that assumed to be due to the deposit of protein, except in *H. S.*, where it falls considerably (1 kg.) short. The recovery phases of the experiments presented attest further to the importance of salt and water in the weight attained, as in each instance weight was lost together with sodium, chloride and water, although nitrogen retention was still going on (*F. R.*, *M. D.*, *N. D.*) or nitrogen losses (as in *H. S.*) were insufficient to account for the decline.

It is of interest to compare the observations in the 3, in which the hypogonadism was of unknown etiology, with those in *H. S.* in which the defect was of pituitary origin. In general the results were the same, with the exceptions that the nitrogen stored was less in *H. S.* and the recovery excretion of nitrogen appeared more quickly and exceeded the baseline much more decisively, and that a slight decline in B.M.R. occurred rather than the slight increase. Whether these peculiarities of *H. S.* depend upon his hypopituitary state, or are variants of the normal influence of testosterone, remains to be determined by examination of a more extended series of patients.

In agreement with the results of the previous study, testosterone propionate produced increased frequency of erections and enlargement of the penis and of the prostate, with a return toward normal during recovery. It only remains to call attention to the fact that in the highly sensitive *M. D.*, plain sesame oil

[5] The assignment of this particular value is made with full appreciation of its highly approximate character. Peters and Lavietes (33) have thoroughly discussed these uncertainties. In any case, we lack information concerning the relative protein and water content of the tissues of the castrate.

alone increased the frequency of erections somewhat, at least as reported by the patient, thus emphasizing further the caution necessary in interpreting this reaction. Studies of the excretion of sex hormones during these experiments will be published later.

SUMMARY

Testosterone propionate, intramuscularly, in 4 eunuchoids, one of which was known to have pituitary disease, produced increased frequency of erections, enlargement of the penis and of the prostate; a consistent decline in urinary nitrogen, reflected in the urea fraction, without evidence of change in the nitrogenous constituents of the blood and amounting to a retention of from 1.16 to 4.51 gm. of nitrogen per day; a decline in urinary sodium, resulting in the retention of from 0.33 to 0.55 gm. per day, usually a retention of chloride; and a gain in weight due largely to the water held in association with the sodium and nitrogen. Creatinuria was diminished in 2 subjects, but the creatinine excretion was not consistently affected. The excretion of potassium declined somewhat in the 2 studied. A slight rise of 6 to 14 points in the B.M.R. occurred in 3 of 4 patients, and the respiratory quotient was unaltered in the 2 studied. Restoration toward normal occurred in all of the affected processes during recovery. Attention is called to the resemblances between the action of testosterone propionate on electrolyte excretion and that of certain of its chemical relatives among sex and adrenal hormones.

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THE EFFECT OF GONADOTROPIC HORMONES ON THE PERSISTING CORPORA LUTEA IN HYPOPHYSECTOMIZED RATS[1]

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In a previous communication (1) it was shown that complete involution of the persisting corpora lutea in hypophysectomized adult rats could be brought about by the injection of the luteinizing hormone (LH) over a period of 10 days. Injections of estrogen, progestin or the follicle-stimulating hormone (FSH) of the hypophysis had no demonstrable effect on these structures. This report will present the results obtained in a further study of the separate and combined action of pituitary FSH and LH and the urinary gonadotropic hormones on the ovaries of hypohysectomized adult rats. Special attention has been given to the behavior of the follicular mechanism, the histological changes in the persisting corpora lutea and the physiological activity of the ovaries as determined by changes in the vaginal smear, the size and appearance of the uteri and the ability to develop placentomata.

Adult virgin female rats were treated with unfractionated pituitary extracts for 5 days prior to the removal of the pituitary gland. A postoperative delay of 10 to 25 days (usually 15) was allowed before any subsequent injections were begun. Fifteen days proved to be sufficient time to allow for involution of all the antra-containing follicles, to establish the fact that the corpora lutea were persisting, and to permit treatment of the animal before cachexia had become unduly severe. Thus the standard experimental animals have been hypophysectomized female rats in which the entire reproductive tracts had become atophic and large persisting corpora lutea plus a few non-antra containing follicles remained in the ovary (fig. 3).

The identification of the original crop of persisting corpora lutea throughout a series of injections was facilitated by the intraperitoneal injection of an intra-vital dye (tryphan blue). This dye is taken up by the corpora lutea only after they have begun to regress (2). Its value, therefore, lies in the fact that it enables one to follow the corpora lutea through the final stages of their involution; the recognition of newly formed corpora lutea is also made easy. However, it became possible, with experience and frequent laparotomies, to make these identifications without the aid of the dye.

The weights of the ovaries were recorded, but these data were not especially informative in some instances since the structural changes occurring within the ovary were of primary concern. Control data were obtained by

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killing hypophysectomized untreated animals at the beginning and at the conclusion of an injection period, but more frequently by removing one ovary at the time treatment was started. The estimation of the weight of the remaining ovary from the weight of the ovary removed involved a standard error of ± 1.56 mg. as calculated from the separate weights of the ovaries of each of 6 rats 15 days after hypophysectomy.

The preliminary treatment with unfractionated pituitary extract was given solely for the purpose of insuring the presence of a greater number of lutein

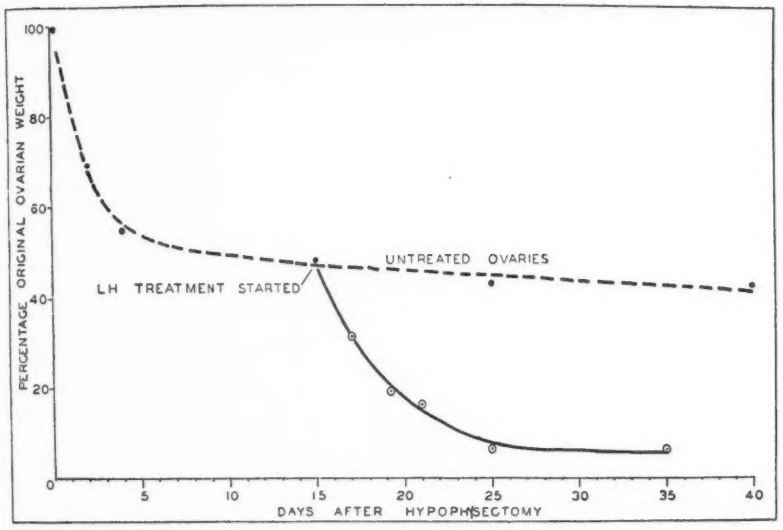

Fig. 1. Showing weight changes in the ovaries of adult rats following hypophysectomy and subsequent treatment with LH.

bodies in the ovaries at the time of hypophysectomy than are normally present. These ovaries contained, in addition to maturing follicles, corpora lutea of ovulation, partially luteinized follicles and some corpora lutea atretica. During the period of postoperative atrophy the ovaries underwent a reduction in size (fig. 1). This occurred most rapidly during the first 5 days, due to the degeneration of macroscopic follicles. Between the 10th and 40th postoperative days the rate of weight loss declined and became almost imperceptible after the 25th day. At any time after the 10th postoperative day the ovaries appeared to contain only persisting corpora lutea on gross examination. These sometimes protruded from the surface of the ovary, giving it the appearance of a loose cluster of lutein bodies.

Anterior pituitary luteinizing hormone (LH)

The luteinizing fraction produced complete involution of the persisting corpora lutea (fig. 2 and 3). These changes were macroscopically apparent by the fourth day of treatment (fig. 14), and proceeded to complete regres-

sion after 10 to 12 days. The rate of regression is accurately reflected in the graph (fig. 1) showing the rate of ovarian weight loss. This rapid regression in ovarian weight is due to the fact that the greater bulk of the ovaries was composed of persisting lutein tissue. The primordial follicles are too few and too small to figure significantly in these regressive changes, although their numbers are obviously reduced. When the injections are continued for 20 days the ovaries become exceedingly degenerate and are always pigmented, having a yellowish brown color. The microscopic picture (fig. 4) shows a dense homogeneous stroma with many small hyalinized areas which probably represent the remains of involuted corpora lutea.

The injections of LH preparations known to be entirely free of FSH did not produce vaginal estrus, and the complete absence of any FSH was clearly demonstrated by the failure of the primordial follicles to develop antra. In other experiments it has been shown that these primordial follicles are remarkably sensitive to FSH stimulation so that the slightest follicular growth can be taken as positive evidence of FSH activity.

Anterior pituitary follicle stimulating hormone (FSH)

The follicle-stimulating fraction has no immediate effect on the number or size of the persisting corpora lutea. During the course of this treatment ovaries were obtained at frequent intervals up to the 12th day, and also at the 20th and 35th days. The first effect is of course the production of antra-containing follicles. These arise on the surface of the gonads and also in between the corpora lutea; the latter often have a polygonal appearance when viewed in cross-section (fig. 5). As the follicles pass maturity the granulosa cells undergo atresia, and the follicles eventually become small thin-walled cysts and disappear without undergoing luteinization (fig. 5). When the FSH injections are continued 20 to 35 days, the ovaries become somewhat smaller but continue to produce estrogen. The persisting corpora lutea, too, show mild regressive changes at these times, but after 10 days of FSH treatment they are not distinguishable from those of untreated control animals. This would seem to show that the destructive action, if any, of FSH on the persisting corpora lutea is of no physiological importance.

Vaginal estrus began on the third or fourth day of FSH treatment and persisted without interruption throughout the course of injections. This in itself cannot be taken as evidence that new lutein tissue did not develop, but it is a safe indication of the failure of any tissue to secrete appreciable amounts of the progestational hormone.

Combination of FSH and LH

When the two pituitary fractions, FSH and LH, were given simultaneously, some rather striking reduplications of the normal cyclic processes were encountered. The following observations are based on data from 48 animals. The macroscopic composition of the ovaries was almost completely reversed within 4 days from a condition showing exclusively corpora lutea to a predominately follicular one (fig. 6) in which the persisting corpora could be

recognized only as pigmented vestiges. This marked structural change can be brought about without altering the weight of the gonads, although they are usually enlarged. That the involution of the persisting corpora lutea occurs more rapidly when LH is acting in conjunction with FSH is evidenced by the fact that the corpora lutea are in a more degenerate condition on the 4th day of the combination treatment than is generally seen on the 8th to

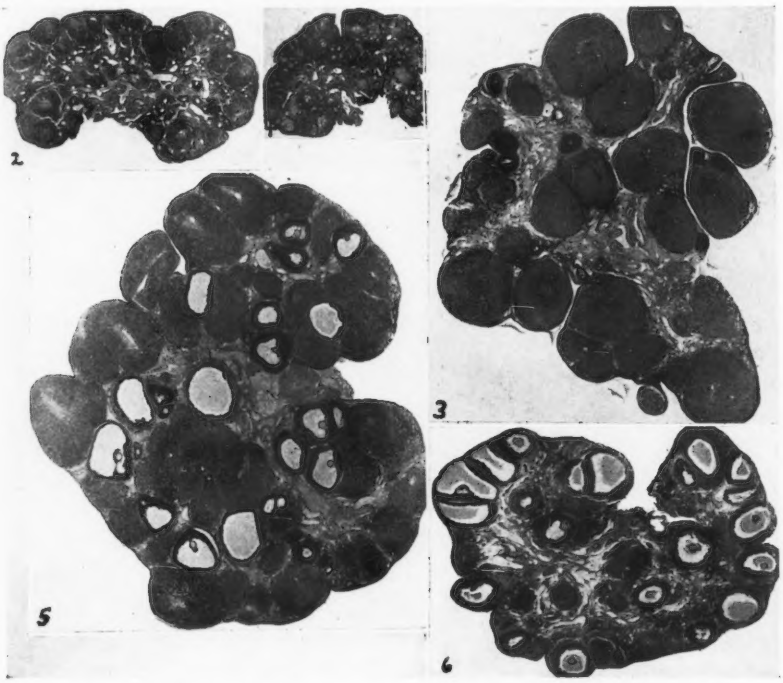

Fig. 2. Ovary of a hypophysectomized adult rat treated with LH for 10 days. Injections started 15 days after operation. x13.
Fig. 3. Control ovary from an adult rat 15 days after hypophysectomy. x13.
Fig. 4. Treated as in fig. 2 for 20 days. x13.
Fig. 5. Ovary of a hypophysectomized adult rat treated with FSH for 7 days. Injections started 15 days after operation. x13.
Fig. 6. Ovary of a hypophysectomized adult rat after 4 days simultaneous FSH and LH treatment. Injections started 15 days postoperative. x13.

10th day of LH treatment. The destructive influence of the combination treatment on persisting luteal tissue was made conspicuously apparent as early as 22 hours by heavy leucocytic infiltration, cytoplasmic vacuolation, nuclear fragmentation, apparent loss of cell walls, and crowding of the nuclei (compare fig. 7, 8 with 9, 10). The rapidity with which these persisting lutein bodies disappear definitely precludes the possibility of this type of treatment stimulating luteal secretion.

Ovaries were obtained at frequent intervals up to the 20th day of treatment with mixtures of FSH and LH, and by correlating their histological structure with the vaginal smear record it was possible to obtain information

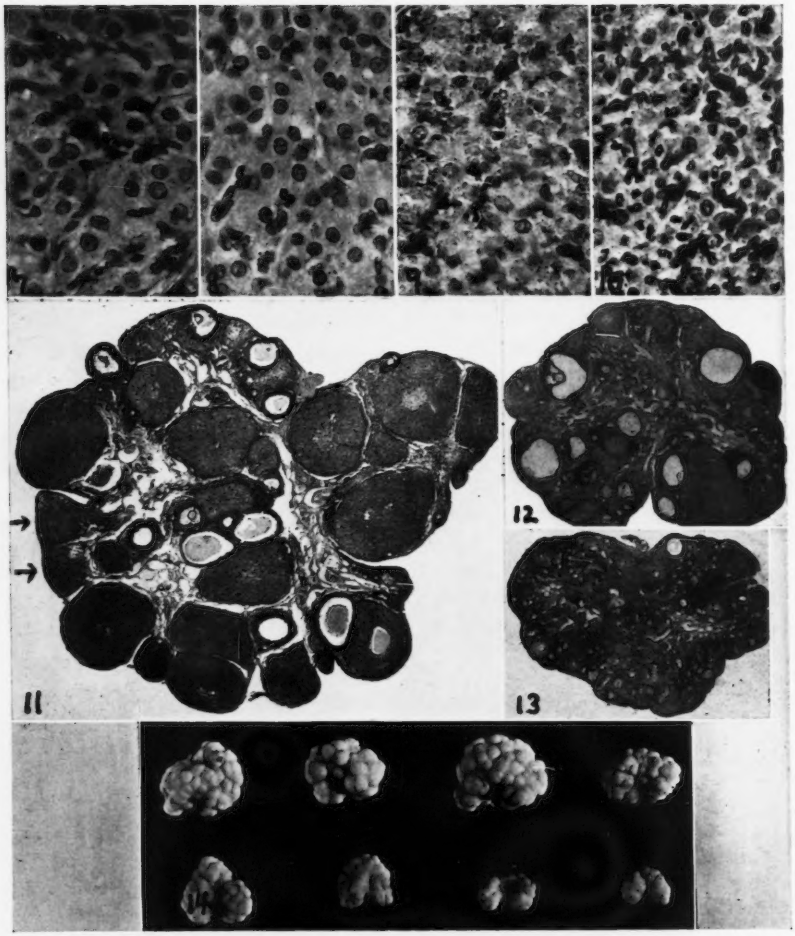

Fig. 7. Corpora lutea of a normal rat after 5 days treatment with unfractionated pituitary extract.
Fig. 8. Persistent corpora lutea 15 days after hypophysectomy.
Fig. 9. Corpora lutea of a hypophysectomized rat 22 hours after treatment with FSH and LH. Injections started 15 days postoperative.
Fig. 10. Treated as in fig. 9 for 48 hours.
Fig. 11. Ovary of a hypophysectomized rat treated with FSH and LH for 6 days. Treatment started 15 days postoperative. Arrows indicate two of the original persisting corpora lutea that are greatly involuted. The prominent corpora are all newly formed. x16.
Fig. 12. Treated as in fig. 11 for 12 days. x16.
Fig. 13. Treated as in fig. 11 for 20 days. x16.
Fig. 14. Upper row shows the control ovaries of adult rats semispayed 15 days after hypophysectomy. Lower row shows opposite ovaries after 4, 6, and 10 days treatment with LH, and one (extreme right) after 10 days treatment with LH and 100 R.U. estrogen daily.

regarding the fate of the original persisting corpora lutea as well as those formed as a result of the treatment. Information was also obtained concerning the successive development of at least 2 fairly distinct crops of follicles. The vaginal smears showed the establishment of an estrous condition on or before the fourth day of treatment. This was followed by a return to di-

estrus within a day or two, and on or near the 10th day estrus was usually re-established, only to be followed by a permanent diestrus from about the 13th day onward.

The ovarian findings show that the first estrus is due to the development of a large crop of healthy follicles (fig.6) which are converted into corpora lutea on the fourth to the sixth day (fig. 11). The second estrus is marked by the disappearance of the first new corpora lutea and the appearance of another and smaller group of follicles (fig. 12) which grow to maturity and induce estrus in spite of the continued presence of LH. Such follicles apparently luteinize only after they have reached a certain size, age or degree of maturity. Beyond the 13th day the further history of these animals on mixed injections is somewhat erratic; follicles and corpora lutea are present for a time and the gonads become progressively smaller. By the 20th day the ovaries are almost always atrophic, weighing 4 to 7 mg. each, contain no follicles and not more than traces of degenerate lutein tissue (fig. 13). Attempts to repair these atrophic gonads have met with poor success. Stopping the LH and continuing with an increased amount of FSH from the same or from a different species source resulted in slight ovarian enlargement and cornification of the vaginal epithelium in 4 out of 5 animals. In one instance only one follicle was visible macroscopically, yet this animal showed an estrous smear. The gonadotropic extracts prepared from pregnant mare's serum and the urine of pregnant women were both ineffective in producing follicular growth or vaginal estrus. These observations are in agreement with the histological finding that by the 20th day of treatment the primordial follicles were either absent or present in very small numbers.

A possible explanation of the ovarian failure following 20 days of simultaneous FSH and LH treatment is that the replacement of follicles does not keep pace with the rate at which they are being matured, the ovaries become exhausted and the last-formed corpora lutea are destroyed by the injected LH. It is also possible that the development of a certain amount of immunity against the injected hormones may be instrumental in causing this ovarian regression. The blood serum however, of such animals after 20 days of treatment does not convey an immunity against the same hormone preparations when they are administered simultaneously to normal 21-day old female rats.

The ability of follicles to grow to maturity and establish estrus in the presence of injected LH, as noted above, can be shown in other ways. For example, when LH is injected into normal adult female rats pseudopregnancy is induced, but it terminates in a return to estrus within 12 to 15 days in spite of the continued daily injection of LH. This of course is brought about through the animal's own pituitary stimulating the growth of follicles. Nathanson and Fevold (3) find that injections of postpartum urine, which apparently contains considerable LH, into adult mice causes a temporary inhibition of the estrous cycles and results in excessive luteinization.

Whether the periodic appearance of diestrus during treatment with mixtures of FSH and LH was due to the active secretion of corpus luteum hormone by newly formed lutein tissue is not known. Suggestive evidence that

the newly formed corpora are non-functional is found in the fact that threading the uteri on the 6th day of treatment did not produce a decidual response. All that can be said at present is that the diestrus coincides with periods of intense luteinization. Had the LH not been present the smear would have remained in constant estrus and the corpora lutea would not have formed. The problem here is the same as that encountered in the normal estrous cycle, i.e., is the diestrus occasioned by the withdrawal of follicular secretion or by active luteal inhibition of the action of estrogen?

Pregnancy urine

In view of the established inability of the urine of pregnancy to repair an atrophic follicular apparatus in the absence of the pituitary gland, and knowing that by the 15th postoperative day there are no follicles present which would be responsive to PU, it was puzzling to find that the injection of this substance always resulted in the establishment of an intense and usually prolonged estrus. The vaginal picture in some of these animals showed a return to the diestrus condition after 5 to 10 days of treatment, but these periods were of short duration. The length of time that these animals will remain in estrus is variable, lasting only 10 days in some animals, but continuing uninterruptedly throughout periods of treatment lasting 50 and 80 days in others.

The persisting corpora lutea usually were not affected adversely by the PU, but if the number of corpora was small and they were in a relatively poor condition at the beginning of treatment, as evidenced by their small size and avascular appearance, the period of estrus did not last more than 10 days, and this was followed by a permanent diestrus. The macroscopic appearance of the persisting corpora lutea has proven to be a good index to their functional capacity. In those animals in which a permanent diestrus was eventually established the corpora lutea were yellowish to light brown in color, and obviously degenerate. This has been interpreted as a condition of secretory exhaustion. When, however, the corpora were present in great numbers and were in good condition at the onset of PU treatment, vaginal cornification and uterine distention was sustained for at least 80 days, at which time the corpora were still discrete bodies and highly vascular although greatly reduced in size.

Histological study of the ovaries obtained after PU treatment confirmed the supposition that follicular development had not occurred, thereby making it impossible to assign the estrogen production to a follicular source. The ovarian interstitial tissue and the persisting corpora lutea offered the only alternatives. Evidence suggesting that the interstitial tissue of the ovary probably does not function in the production of estrogen was obtained from several different experiments, and it has often been stated in the literature that the ovarian interstitial cells are of dubious functional significance. Conclusive evidence that the interstitial cells may be eliminated from consideration as the source of estrogen during PU treatment was obtained from the following experiment. Four hypophysectomized adult female rats with persisting corpora

lutea were treated with LH for 4 days to damage the corpora (but not the interstitial cells), and then given PU for 10 days. Estrus did not occur. The interstitial tissue in these animals was found to be in a stimulated condition, but the uteri remained thread-like, and their vaginae did not become cornified. Furthermore, ovaries obtained after 30 days' treatment with PU show the interstitial tissue in a state of regression, yet the estrous condition may continue for a much longer period. The injections of LH also produced repair of the interstitial elements, but estrus was never observed during this type of treatment. The above experiment, in which estrus failed to appear when PU treatment followed partial involution of the corpora lutea induced by LH action, also shows with considerable conclusiveness that the lack of estrogen secretion was due specifically to the fact that the persisting corpora lutea were functionally incapacitated.

Estrogen

Injections of 40 R.U. estrogen daily did not alter the size or histological structure of the persisting corpora lutea in hypophysectomized rats (1). Smith (4) also mentions that estrogen has no effect on the appearance of these structures. Some recent reports, however, have re-opened the problem of a possible stimulative action of estrogen on the corpora lutea. Westman and Jacobsohn (5) find that estrogen will prolong the functional life of the corpora lutea of pseudopregnancy in hypophysectomized rabbits, and this remarkable effect has been confirmed by Robson (6). Selye and Collip (7) report that the injection of 200 γ of estrone along with unfractionated pituitary extract into hypophysectomized rats gave a better ovarian hypertrophy than did the pituitary extract alone. They also state that it was evident that the corpora lutea were larger in the animals receiving both estrin and pituitary extracts. Identical treatment of normal animals by the same authors confirmed the observations made by Wolfe (8) that estrogen in the normal adult female rat results in the production of enlarged corpora lutea typical of those seen in the late stages of pregnancy.

The data obtained in the following experiments failed to show a beneficial action of estrogen on the ovaries of hypophysectomized rats. Eighteen adult female rats were hypophysectomized and 9 were started immediately on FSH and LH, whereas the remainder were given the same dosages of FSH and LH plus 200 R.U. of estrone daily. The injections were continued for 10 days, during which time the animals receiving both estrone and pituitary hormone remained in constant estrus, whereas those receiving only the pituitary hormones showed a variable period of diestrus between days 5 and 8. At autopsy on the 10th day the ovaries of the estrogen-pituitary group weighed 85 to 160 mg. (average 117 mg.), whereas those in the pituitary group weighed 93 to 152 mg. (average 122). The relative size of the corpora lutea in the two groups of rats was obtained by the method described by Wolfe (8). In the estrone-pituitary group the mean product of the two greatest diameters of the corpora was 1.07 mm., and where no estrone was present it was 1.02 mm. An additional opportunity for demonstrating an action of estrogen on the corpora lutea lay in the possibility that estrogen may afford them protection

against the destructive action of LH. The injection of 100 R.U. estrone daily along with a known effective dose of LH into each of 4 rats beginning 15 days after hypophysectomy resulted in as pronounced involution of the persisting corpora after 10 days as was obtained with the LH alone (fig. 14).

The question which immediately arises with regard to the persisting corpora lutea is whether they can be made to secrete luteal hormone. The problem was made difficult by the lack of a suitable end point for progesterone action in the rat. It is well known, however, that corpus luteum hormone (progesterone) will inhibit the estrous cycle for a limited period (1, 3), and that estrus does not occur during periods of pronounced luteal activity such as pregnancy and pseudopregnancy. The injections of estrogen into normal rats during pseudopregnancy and lactation have been phenomenal in their ineffectiveness to sustain an estrous vaginal reaction (8, 9, 10). Any pertinent experiment on hypophysectomized rats in postoperative atrophy must involve some estrogen therapy because of the necessity of restoring the reproductive tract to an estrous condition in order to test for a possible inhibiting or modifying influence produced by the corpus luteum hormone. In 4 hypophysectomized (15 days) adult rats with persistent corpora, 2 R.U. of estrogen was injected daily for 3 days prior to the beginning of an LH treatment and was continued at that dosage during the LH injections (7 days). The LH treatment did not interrupt the constant estrous smear induced by the injected estrogen. In a similar experiment one R.U. of estrogen was given on the day following the beginning of LH treatment. This single injection of estrogen produced vaginal estrus in 72 hours, showing that luteal secretion apparently had not been induced by the LH.

The ability of PU to sustain a state of pseudopregnancy following extirpation of the hypophysis was tested in the following manner. Five rats receiving 10 R.U. of PU daily were hypophysectomized on the 5th day and their uteri traumatized. The PU treatment was continued until autopsy 4 days later, at which time estrus had supervened and precluded the development of a decidual reaction. The estrogenic hormone in question was presumably produced by luteal tissue, since the follicles present at the time of hypophysectomy underwent rapid thecal luteinization as determined by studying single ovaries removed at laparotomy 48 hours after pituitary ablation.

Some further experiments have contributed support to a conviction that in non-pregnant rats the corpora lutea persisting after hypophysectomy are totally non-functional. Threading the uteri of 5 rats after a previous 5-day estrogen treatment did not result in the formation of placentomata. The estrogen injections (10 R.U. daily) were begun on the 10th postoperative day. It also seemed worth while to test the possibility that the corpora lutea might gradually assume secretory function during a more prolonged estrogen treatment and cause an interruption of the estrous smear. Such an interruption, however, was not witnessed when 2 R.U. of estrogen were injected daily for 20 days beginning on the 15th day after hypophysectomy. In 4 additional rats the hypophyses were removed and the uteri traumatized on the fourth day after cervical stimulation (stimuli applied during estrus). The decidual reaction was negative in 3 animals, and made only a poor start in the fourth.

DISCUSSION

The factors which limit the life span of the corpora lutea have received but scant attention. Most of the information relating to this problem has been gained through surgical methods (4, 11, 12, 13). In the present investigation, as in a previous communication (1), advantage has been taken of the opportunity of studying the influence of several hormone preparations on the corpora lutea persisting in hypophysectomized rats in the absence of gross follicles. Although these structures are non-functional they are, at least morphologically, quite normal (fig. 8). The only substance found, which by itself will induce histological involution of such corpora, paradoxically has turned out to be the same substance (LH) which under proper conditions acts as a stimulus for their formation. It is difficult at present to fit this observation into the current conceptions of the physiological processes occurring in a normal reproductive cycle.

When the two pituitary preparations (FSH and LH) were given simultaneously, involution of the persistent corpora was remarkably rapid. If, as the available evidence (14, 15, 16) suggests, the activity of the pituitary gland fluctuates during the cycle there exists a possibility that when the output of FSH and LH reaches a certain balance sometime prior to estrus the corpora lutea of the last cycle are affected adversely. It is important to note that the corpora lutea formed in hypophysectomized rats as a result of simultaneous treatment with FSH and LH were presently destroyed in the same manner as were the corpora which had been persisting for at least 15 days. It is unfair then to assume that the persisting corpora are in some way abnormally susceptible to destructive stimuli.

In regard to the limited number of apparent estrous cycles which occurred during the simultaneous administration of FSH and LH one must consider that the balance of the two hormones was probably quite abnormal, and that they were present at all times in the same concentrations. The wonder is that the ovaries exhibited any attempt at a rhythmic function.

The marked difference in the action of LH and of PU on the structure and function of corpora lutea persisting in hypophysectomized rats is additional evidence of the fundamental dissimilarity between these two gonadotropic substances.

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GONADOTROPIC ACTION OF NORMAL MALE URINE EXTRACT ON THE DOG

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The presence has been suggested (1, 2, 3, 4), of a gonadotropic substance in normal male urine, comparable to the anterior-pituitary-like follicle-stimulating hormone found in menopause urine. Follicle stimulation in immature rats has been produced with this extract (5) as well as an increase in the weight of the mouse uterus (6). In both instances the action of the extract was comparable to menopause or castrate urine extract. However, Katzman (7), while advocating that gonadotropic substances can be obtained from the urine of normal males, was not able to produce ovarian enlargement with his extract. Furthermore, the urine of males and females with essential hypertension has been found to contain a prolan-A-like substance which causes hypertrophy of the uterus and ovary in the rat, whereas normal urine gave only moderate responses, and it was concluded that, if present at all, the quantity was very small (8). Extracts of the urine of male castrates (9) and young men with vegetative disturbances (10) also exhibit an activity comparable to menopause urine extract.

It appeared of interest to test an extract of normal male urine on the dog and compare the results with those previously obtained with an extract of menopause urine (11).

RESULTS

External signs of estrus. Seven mature dogs in the anestrous phase of their cycle were injected daily until the first day of estrus. An immature animal was also injected until signs of estrus appeared. Vulval swelling occurred in every case and seemed to reach a maximal state. Hemorrhagic discharge, usually evident shortly after the first vulval swelling, was observed for a period ranging from 2 to 10 days. One dog, *3*, exhibited only slight bleeding in 2 days. In spite of the small amount of bleeding in dogs, *3*, *9*, and *10*, the duration of the proestrous period was actually 5 to 7 days in length, as shown by vaginal smears from the time of the first vulval swelling until the time of estrus.

It was noted, however, that neither the amount of bleeding which occurred nor its duration nor the appearance of a completely cornified smear was reliable as an index of willingness to copulate with the male. In two instances (table 1), the external signs of estrus were all present but these animals would not accept the male. In 2 other cases in which bleeding lasted for only 2 and 3 days, copulation occurred daily for 10 and 13 days respectively.

TABLE 1. EFFECTS OF NORMAL MALE URINE EXTRACT PROSPERMIN ON THE ESTROUS REACTIONS OF THE DOG

Dog	Initial phase of cycle	Weight	Period injected	Total R.U. injected	Beginning vulva swelling	Duration of bleeding	Mating, time from 1st inj.	Remarks
No.		kg.	Days		Day	Days	Days	Sacrificed
Dogs injected until the first day of estrus								
2	Immature	10.4	17	1494	10th	10	—	26th day of experiment
3	Anestrus	11.6	14	1758	11th	slight	14–21	4th day of estrus
4	Anestrus	12.5	11	2200	7th	6	—	End of estrus
6	Anestrus	8.9	14	1008	11th	5	17–23	84th day of experiment
7	Anestrus	12.0	15	1440	12th	9	17–25	Pregnant: not sacrificed
9	Anestrus	9.2	10	745	7th	2	11–20	26th day of metestrus
10	Anestrus	11.3	10	895	6th	3	13–25	21st day of metestrus
8	Pregnant	8.6	18	1890	13th	6	—	23rd day of experiment
Dogs injected until proestrus								
5	Anestrus	15.8	8	1024	10th	5	16–	2nd day of estrus
12	Anestrus	15.2	12	1180	10th	6	—	40th day of experiment
			15[1]	1870	—	—	—	
13[2]	Anestrus	12.4	10	990	10th	8	18–	4th day of estrus
Dogs which failed to respond								
1	Castrate	8.6	22	1890	—	—	—	26th day of experiment
11	Anestrus	7.3	23	1820	—	—	—	34th day of experiment

[1] Second series of injections after 7-day interval.
[2] Dog received 500 R.U. follutein in the first day of estrus.

One dog, found later to be in midpregnancy at the time injections were started, responded by showing all the external signs of estrus: swelling of vulva, external hemorrhagic and cornified smears. This animal would not accept the male, however. At autopsy 6 well-developed embryos were undergoing resorption and large follicles were observed on the ovaries.

A group of 3 dogs in the anestrous stage were injected until the first signs of proestrus were obtained, when the injections were discontinued. Two of these animals went through an apparently normal proestrous phase into the estrous phase. Bleeding occurred and the smear showed cornified cells only. Both accepted the male.

The third animal showed vulval swelling and hemorrhagic discharge. The smear, however, never became fully cornified and the animal refused the male. After a 7-day interval, during which time the smear filled with leucocytes and the vulva regressed, a second series of injections was started and continued for 15 days. No external signs of estrus or smear changes resulted.

In order to determine what influence this extract might have on the uterus and vagina directly, a castrate dog was injected daily for 22 days with a total of 1890 R.U.[1] of the hormone. No external signs of estrus or proestrus appeared. The vaginal smears remained unaltered.

[1] One rat unit is the minimum amount which, when injected subcutaneously twice daily for 3 consecutive days in a 30-day-old female rat, produces only large follicles at the end of 100 hours after the first injection.

Histological changes. Three dogs were sacrificed during estrus, and the endometrial development studied. In early estrus (dog *5*), the endometrium was not greatly hypertrophied, but the basal glandular condition was more pronounced than in the anestrous state. The immature dog, *2,* in midestrus exhibited an endometrium of an early estrous development. The uterus in both cases was hypertrophied, due to an increase in size of the myometrium and the stroma. Dog *4* was sacrificed 15 days after the first appearance of proestrous bleeding and on the first day that leucocytes appeared in the smear, both of which seemed to indicate the end of estrus. The uterus was small since neither the myometrium nor the stroma was hypertrophied, but the endometrium was of midestrous development. The increase in the basal glands was evident (fig. 4). The endometrial development, in each case, seemed to lag slightly behind the condition observed in animals at the same stage of spontaneous estrus, or estrus induced with menopausal urine extract.

Two dogs, *9, 10,* were sacrificed on the 26th and 21st days of pseudopregnancy, respectively. The endometrium in both cases was undergoing regression, but gave evidence of a previously existing typical glandular condition for this period. No dogs were sacrificed earlier than the 21st day of metestrus, and thus the degree of glandular complexity normally attained in early pseudopregnancy was not ascertained. At the end of pseudopregnancy the endometrium had regressed to an anestrous condition.

The ovaries clearly demonstrated the presence of a follicle-stimulating substance. In two instances the weight of the ovaries was significantly increased, as they averaged 3.3 and 4.7 gm., or 6 to 8 times the weight of the normal anestrous ovary. In spite of this excessive follicle stimulation the ovarian weight returned approximately to normal after 60 days.

Four dogs were sacrificed during estrus. The ovaries of a mature dog, *5,* in estrus exhibited pronounced follicle stimulation in which many follicles responded, but all to fairly equal size (fig. 1). In a few instances there were indications of luteinization. At the end of estrus (dog *4*) there were very few follicles present, as virtually all the ovarian tissue was composed of corpora lutea which, at this time, were already compactly formed, as shown in figure 2. The ovary of the immature dog, *2,* exhibited a definite response to the follicle-stimulating action of the extract. By sectioning longitudinally, one ovary was found to be composed entirely of 3 large follicles, virtually in exclusion of stroma and with no thickening of the granulosa. The other ovary consisted of one large follicle. Normal ova were present. The greatest follicle stimulation as to size of the individual follicles was observed in the ovaries of the pregnant dog, *8,* as shown in figure 3. These follicles exceeded those observed following stimulation with menopausal urine extract. However, the presence of some luteinizer in the extract was revealed by the thickening of the granulosa.

Two dogs were sacrificed during pseudopregnancy. On the 21st day of this period (dog *10*) there was evidence of regression in the general ovarian condition, as indicated by the smaller corpora and the presence of more stroma. Dog *9,* sacrificed on the 26th day, exhibited ovaries with little stroma

Fig. 1. Ovary in induced estrus showing follicle stimulation. x6¾.
Fig. 2. Longitudinal section of an ovary at the end of estrus, showing the many corpora lutea. x6.
Fig. 3. Stimulation of follicles in the ovary of a pregnant dog. x5¼.
Fig. 4. Endometrium at the end of estrus showing the increase in the basal glands. x14.

PROTOCOLS

Dog *1*. Mature. Castrate. Injections started 2/8/37. In 20-day period 17 daily injections of 70 R.U. given. On 2/28, 140 R.U. per day, and for 5 days. Sacrificed 3/5. No effect.

Dog *2*. Immature (8 months). Injections started 2/8/37. In 21 days 16 daily injections of 83 R.U. and one of 166 R.U. On 2/20 bleeding and vulva swelling. On 2/24 cornified smear. On 2/28 bleeding stopped. On 3/1 sacrified in estrus. Uterus hypertrophied. Ovaries averaged 1.45 gm. Follicles. No ovulation points.

Dog *3*. Mature. Anestrus. Injections started 2/19/37. In 14 days 9 daily injections 92 R.U. and 5 injections 186 R.U. 3/1 vulva swelling and bleeding. Slight bleeding, 3/2, smear cornified. Mated 3/4. End of estrus, 3/12. Sacrificed, 5/12. Not pregnant. Uterus small. Ovaries .48 gm.

Dog *4*. Mature. Anestrus. Injections started 3/2/37. Daily 11 injections of 200 R.U. Vulva swelling and bleeding 3/8. Cornified smear 3/10. No bleeding 3/13, smear cornified; 3/23 first leucocytes in smear. Dog never mated. Sacrificed. Uterus small. Ovaries covered with corpora 1.6 gm.

Dog *5*. Mature. Anestrus. Injections started 3/8/37. Daily 8 injections of 128 R.U. Vulva swelling and bleeding 3/17. Bleeding stopped 3/21. Mated 3/23, sacrificed 3/24. Uterus hypertrophied. Ovaries 3.3 gm. Many follicles. No ovulation points.

Dog *6*. Mature. Anestrus. Injections started 3/30/37. Daily 14 injections of 72 R.U. Vulva swelling and bleeding 4/10. Cornified smear 4/13. On 4/15 bleeding had stopped. Mated. On 4/22 would not mate. On 6/21 sacrified. Not pregnant. Ovaries 0.7 gm.

Dog *7*. Mature. Anestrus. Injections started 3/30/37. Daily 15 injections of 96 R.U. Vulva swelling and bleeding 4/11. On 4/15 mated. Last day bleeding 4/19. On 4/23 last mating. Laparotomy 5/12. Pregnant, two embryos. Delivered, 6/18, two pups. Pups weaned 7/14.

Dog *8*. Mature. Mid-pregnancy. Injections started 3/30/37. Daily 18 injections of 105 R.U. in 21 days. On 4/16 vulva swelling and bleeding. On 4/20 cornified smear. Sacrificed. Six embryos undergoing resorption. Ovaries 4.7 gm. Many large follicles.

Dog *9*. Mature. Anestrus. Injections started 5/29/37. Daily 9 injections of 75 R.U., and one of 70 R.U. in 11 days. On 6/4 vulva swelling and bleeding. On 6/7 no bleeding. On 6/9 mated. On 6/17 last mating. On 7/14 sacrificed. Not pregnant. Uterus hypertrophied. Ovaries 1.37 gm.

Dog *10*. Mature. Anestrus. Injections started 5/29/37. Daily 9 injections of 95 R.U., and one of 85 R.U. in 11 days. On 6/3 vulva swelling. On 6/5 bleeding. On 6/7 no bleeding. On 6/10 mated. On 6/22 last day mating. On 7/14 sacrificed. Uterus hypertrophied. Ovaries .65 gm.

Dog *11*. Mature. Anestrus. Injections started 11/11/37. Daily 7 injections of 60 R.U., one of 50 R.U. and 15 of 90 R.U. in 31 days. On 11/14 sacrificed. No effect.

Dog *12*. Mature. Anestrus. Injections started 11/8/37. Daily 3 injections of 90 R.U., 9 of 100 R.U. in 15 days. On 11/17 vulva swelling. On 11/19 bleeding. On 11/25 bleeding stopped, smear not completely cornified, and would not mate. On 11/27 vulva smaller and leucocytes in smear. On 11/29 second series of injections begun. One of 125 R.U., 3 of 165 R.U., 10 of 120 R.U. and one of 50 R.U. in 18 days. On 12/16 sacrificed. No return of estrus. Uterus small. Ovaries .4 gm.

Dog *13*. Mature. Anestrus. Injections started 11/10. One of 90 R.U. and 9 of 100 R.U. in 12 days. On 11/19 vulva swelling and bleeding. On 11/24 smear cornified. On 11/26 bleeding stopped. On 11/27 mated. Injected with 500 R.U. follutein. On 11/30 sacrificed in estrus. Uterus hypertrophied. Ovaries had 6 ovulation points.

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and large corpora, but the corpora were extensively vacuolated. At the end of metestrus, the ovaries had a few very small corpora lutea, many primary and growing follicles and, in general, exhibited a condition similar to the normal at this phase.

Ovulation points were not observed on the ovaries of the dogs sacrificed during estrus, and therefore pregnancy was employed to determine this point. Five mature dogs were brought into estrus and bred repeatedly with normal males. Two dogs, *3, 6*, were kept in the laboratory for 63 days but failed to deliver and showed no evidence of having been pregnant. Laparotomies were performed on 3 animals, and in two cases pregnancy had not resulted, but the third (dog 7) was found to be pregnant and on the 56th day of metestrus

delivered 2 pups. The pups were suckled by the mother for a month and remained in the laboratory for 2 months longer, being normal in every respect.

In view of the failure to obtain pregnancy in 4 cases and the small litter in the other instance, more luteinizing hormone, in addition to that present in the extract, would seem necessary to induce ovulation in these animals. Therefore, one experiment dog (*13*) was attempted to study this point. The dog was brought into estrus with normal male urine extract and on the first day of estrus was injected intraperitoneally with 500 R.U. of follutein. On the fourth day of estrus 6 ovulation points were found. The ovaries were comparable to those of spontaneous estrus in that a normal number of follicles had ovulated and the ovulation points were well spaced. No large follicles remained. The uterus was hypertrophied and the endometrium was of an estrous type. Further experiments will be necessary to determine whether ovulation can be induced in every instance by this method.

DISCUSSION

The presence of a gonadotropic substance comparable to the FSH of the anterior pituitary has been demonstrated with an extract of normal male urine, 'Prospermin', in its effects on the female dog. This extract, although apparently not entirely free of luteinizing substance, was similar in its action to that of menopause or castrate urine extract. The FSH-like action was exhibited by the induction of estrus in the normal dog, in causing follicle stimulation and resorption of the embryos in the pregnant dog, and in its failure to affect the castrate dog.

This male urine extract induced vulval swelling, proestrous bleeding and estrus followed by a metestrous period. Vaginal smear changes were typical in most respects. The proestrous period was of slightly shorter duration than normal. Furthermore, 2 dogs on the lowest total dosage exhibited only slight bleeding. The estrous period was of normal duration being comparable to estrus induced with menopausal urine extract.[2] In both cases the duration was judged by the acceptance of the male.

In general, a larger number of rat units of normal male urine extract was necessary to induce estrus than were necessary with M.U.E. However, there appeared to be no correspondence between the total dosage and the time of the initial reaction. Two of the 3 cases in which administration of the hormone was discontinued during proetrus progressed through the cycle into the estrous phase. Injections of M.U.E. until proestrus was established was always followed by estrus, and appeared the most satisfactory method to avoid overdosage as much as possible.

In estrus, the endomentrium appeared to lag slightly in comparison with spontaneous estrus, whereas with M.U.E. the endometrium followed the normal very closely. The ovary of the mature dog in estrus was stimulated in the same manner as with M.U.E., in that many follicles responded. The immature dog ovary also compared with the M.U.E. effect in that few follicles responded but were greatly stimulated. The ovary at the close of estrus exhib-

[2] Hereafter designated as M.U.E.

ited many fully-formed corpora, which is a more advanced condition than found normally, but this may possibly have been the result of a large dosage, and of being administered in a short time as well as the presence of some luteinizer in the extract. Vacuolation of the corpora was observed in one instance in pseudopregnancy on the 26th day, which would seem to shorten this phase.

Earlier experiments demonstrated that ovulation rarely, if ever, occurs following injection of M.U.E. alone (11). Ovulation points were not found on the ovaries during estrus induced with male urine extract so that pregnancy was used to determine this point. In one case the dog became pregnant, delivered and suckled 2 normal pups. However, 4 other dogs on the same test failed to become pregnant which seemed to indicate that sufficient luteinizer was not present in the extract to allow ovulation in all cases. Therefore one experiment was performed in which follutein was injected on the first day of induced estrus and 6 ovulation points were found on the ovaries on the fourth day of estrus.

Dicker (8) was unable to find appreciable quantities of prolan-A in normal urine, but did find this material in the urine of males and females with essential hypertension. However, large amounts of FSH-like material are present in normal male urine, as shown in these experiments which also support the previously reported gonadotropic action of normal male urine (1 to 6).

SUMMARY

An extract of normal male urine was found comparable to menopause urine extract in inducing estrous reactions in 11 of the 12 normal dogs tested. Other indications of gonadotropic activity in the manner of a follicle stimulator were ascertained by excessive follicle stimulaton, in which the ovary increased in weight to as much as 8 times that of a normal anestrous ovary, the resorption of embryos in a pregnant animal, and the failure to affect a castrate dog. Normal male urine extract was found capable of inducing all the external manifestations of estrus, namely, vulval swelling, hemorrhagic discharge, and estrus as determined by acceptance of the male. In addition, endometrial hypertrophy and vaginal smear changes also resulted. Some specific differences are cited in comparison with spontaneous estrus, and estrus induced with menopause urine extract. Ovulation was determined by pregnancy in which 1 of the 5 dogs tested became pregnant, delivered and suckled 2 normal pups. Injection of follutein on the first day of estrus caused ovulation in the one case tested.

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THE EFFECT OF CASTRATE URINE HORMONE ON THE TESTIS

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The effect of the hormone obtained from the blood and urine of menopausal or castrated women (1, 2) is so strikingly follicle-stimulating that at present many speak of this substance as the 'follicle-stimulating hormone'. Since the work of Smith, Engle and Tyndale (3) showed that this substance maintained the tubular portion of the testis of the hypophysectomized rat, it became interesting to study its effect on the intact animal in order to note what additional influence it might have on normally developing testis.

For this purpose, 40 albino rats of Wistar Institute strain, ranging from 30 to 108 days of age, were used. These were divided into 2 groups of 20 each, according to sexual maturity. Thus *group 1* consisted of animals older, and *group 2* comprised rats younger, than 45 days, the age at which normal testicular descent generally occurs (4). Half of each group was used for test purposes, while the other half served as litter-mate brother controls. All animals were kept under similar conditions, having food (Purina dog-chow) and water constantly present.

The test animals received 10 daily (except Sunday) intraperitoneal injections of 10 R.U. of the hormone for 10 days (totaling 100 R.U.). Control animals received no injections. The hormone used represented the water-soluble fraction of menopausal or castrate urine, and had been assayed and standardized for its follicle-stimulating effect upon the hypophysectomized rat, so that each cubic centimeter contained 25 R.U.

Twenty-four hours after the last injection all animals were sacrificed, body and testicular, including epididymis weights were recorded and averaged, and probable errors were calculated for the means. Results obtained for the test animals of each group were then compared with the respective control group, and the differences noted were computed as ratios (deviation $\pm$ probable error of deviation). A ratio of 3 was considered as critical, so that differences observed were considered as probably significant only if their ratio equalled or exceeded this figure.

After gross studies were made, the testes including the epididymes were fixed in formalin and stained with haemotoxylin-eosin for microscopic survey.

RESULTS

It was noted grossly that on the fourth day the immature test animals showed testicular descent, whereas the controls failed to show this phenomenon.

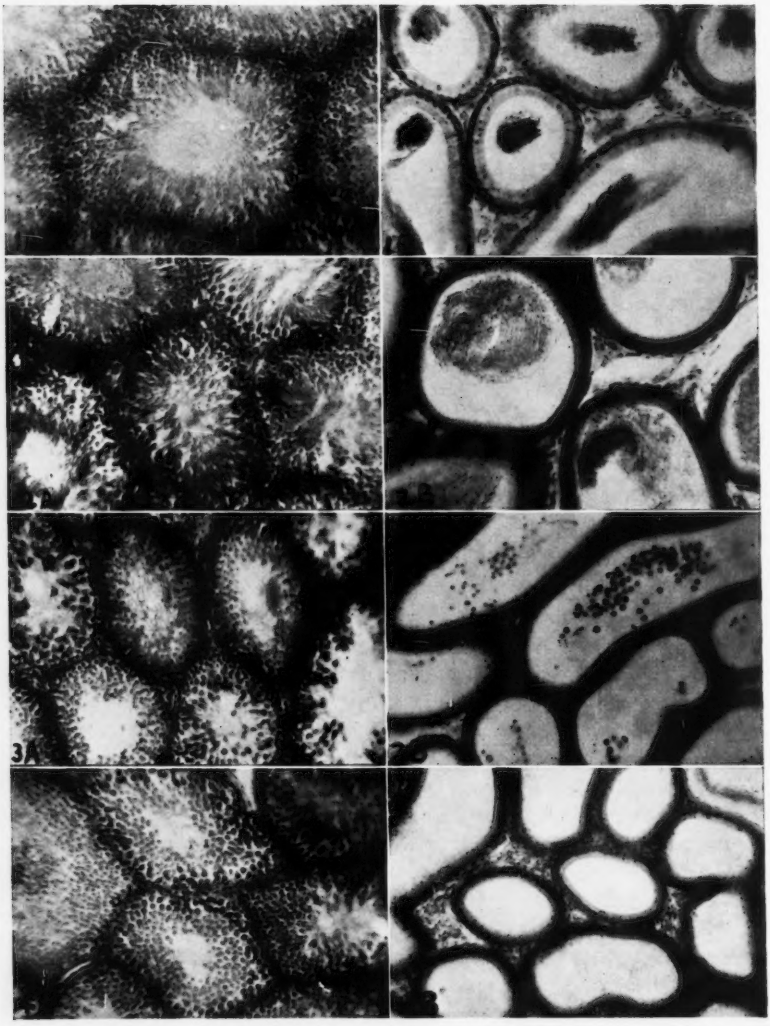

Fig. 1*A*, Testis of adult test rat showing increased tubular diameter and moderate increase in interstitial tissue; 2*A*, Testis of adult control rat showing complete spermatogenesis; 3*A*, Testis of immature test animal showing marked interstitial tissue increase and mitotic forms reaching to the tubular lumen; 4*A*, Immature control testis. Spermatogenesis incomplete. The mitotic figures do not reach the tubular lumen.
1*B*, Epididymis of adult test rat showing marked clumping of spermatozoa; 2*B*, Epididymis of adult control animal; 3*B*, Epididymis of immature test rat showing numerous mitotic figures and premature spermatozoa within the lumens; 4*B*, Epididymis of immature control rat with empty tubules.

At the time of sacrifice, the older test animals possessed body weights which averaged 176.7 ± 10.4 gm. as compared with 167.9 ± 7.0 gm. for the controls. The difference noted (8.8 ± 12.2 gm.) led to a ratio of 0.72.

The testicular weights of this test group averaged 2.8027 ± 0.6312 gm. as compared with 2.3972 ± 0.260 gm. for the controls. The difference of

0.4055±.0406 gm. in favor of the test group led to the high ratio of 9.99.

In the case of the younger animals, body weights of the test animals averaged 45.2±2.3 gm., as compared with 51.6±2.9 gm. for the controls. The difference here of 6.4±3.7 gm. gives a ratio of only 1.73.

Testicular weights for this group averaged 0.6980±0.0319 gm. for the test animals, and 0.3599±0.0314 gm. for the controls. The difference of 0.3381±0.0447 gm. in favor of the test animals leads to a ratio of 7.56.

Microscopically (fig. 1-4A), the testes of the adult test rats showed a tubular diameter which was greater than the normal and a moderate increase in interstitial tissue. Complete spermatogenesis was present in all of the mature animals.

The testes of the immature group showed more striking results. The interstitial tissue response in this group was more marked than that in the mature animals. In addition, the tubular epithelium appeared to be in a more actively proliferative stage, showing many mitotic forms and numerous secondary spermatocytes. A few spermatids (sperm-heads of Moore, 5) were noted in the test group but these were also seen, in lesser numbers, in the controls. The proliferative activity of the germinal epithelium could best be appreciated from a study of the epididymes (fig. 1-4*B*), which in the adults showed a greater clumping of the spermatozoa in the test animals than in the controls. In the immature control group, the tubules of the epididymes were empty (fig. *4B*), whereas in the test animals numerous mitotic spermatocytes were seen (fig. *3B*).

DISCUSSION

From the results obtained, it appears that castrate urine hormone is not only follicle-stimulating but has a striking effect on the male. Here it tends to increase significantly the size of the mature and immature rat testis. Although this increase in size is relatively greater in the younger, the tubular diameter becomes greatest in the mature group.

In its induction of premature testicular descent, and in its stimulating effect upon the interstitial tissue, this hormone bears striking resemblance to the pregnancy urine extract (6, 7). This result in the normal rat is interesting, since it has been shown by Smith, Engle and Tyndale (3) that the hypophysectomized rat responds with marked proliferation and maturation of germ cells, but no response in the interstitial tissue or accessory genital organs. The added effects noted in the experiments herein reported lead one to assume that they are due to the animal's own hypophysis. If this is true, it means that castrate urine hormone also stimulates the pituitary gland, since these secondary changes are greater than those found in the normal rat.

The stimulating effect of this hormone on the germinal epithelium is very marked, but it is important to note that although germ cells proliferate more rapidly there is no indication of hastened maturity. This is best indicated by studying the testes of the immature animals which shows both test and control testes to be in the sperm-head stage (32 day-old animals). Further evidence of lack of maturation is adduced by the contents of the immature epididymi. Since there are those who believe this hormone to arise in

the pituitary gland, it is interesting that it possesses no growth-stimulating effect.

That this is not due to a toxic factor may be gathered from the fact that toxins usually stunt growth. In the animals studied, no stunting was observed.

SUMMARY

Follicle-stimulating hormone obtained from menopausal and castrate blood and urine induces premature testicular descent in normal immature albino rats. It increases the weight of the testes significantly in both mature and immature animals, although the effect is greater in the younger group. Interstitial tissue is increased in old and young rats, but is greater in the latter. Tubular diameter is increased in the adult testis. Increased germinal cell proliferation results from the use of this hormone, as noted from increase in mitotic forms in the testes, and the finding of cells in the immature test epididymis tubule when none are seen in the control. The epididymis in this respect may serve as an index to testicular function. Although germinal cell proliferation is increased, maturation is not hastened. Follicle-stimulating hormone has no effect on general body growth.

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ADIPOSITY AND DIABETES MELLITUS IN A MONKEY WITH HYPOTHALAMIC LESIONS[1]

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In nearly 50 monkeys and in over 300 cats lesions have been placed in the hypothalamus, and in only one animal, a monkey, has a definitely pathological adiposity been observed. In this monkey the adiposity was associated with diabetes mellitus and a moderate disturbance in the capacity to regulate body temperature. Several of the cats which developed diabetes insipidus also became obese, but not more so than control cats kept in small cages and given a generous diet.

CLINICAL COURSE

An immature male Macacus Rhesus monkey, weighing 2.6 kg. and having a crown-rump length of 35 cm. on the day of operation, had been in the laboratory for about 2 months, and had shown no abnormality sufficient to attract attention. It was well nourished but not fat, its weight being only a little above the average for its length and both corresponding to an age of from 24 to 30 months according to the table given by Hartman and Strauss (1). The operation was performed on May 28, 1935. Lesions were placed bilaterally in the hypothalamus with the aid of the Horsley-Clarke stereotaxic instrument. A unipolar electrode was used, the indifferent electrode being in the rectum. One puncture was made on each side 2.5 mm. from the midline, both being in a frontal plane 9 mm. anterior to the interaural line. Three lesions, centered 1 mm. apart, were made in the hypothalamus along each puncture. Each lesion was produced by a direct current of 3 milliamperes passing for 60 seconds. The animal recovered promptly from the operation, and was fed the same diet as the other monkeys of the same series, a diet consisting of potatoes, carrots, fruit, corn, oats, bread and milk. All of the monkeys had as much as they would eat but none of the others became obese.

Adiposity. About 3 weeks after the operation it was noted that the monkey had become quite fat and had developed a voracious appetite. On weighing the animal a gain in weight of 1 kg. was found. From this time on, the gain in weight was rapid. Two months after operation the weight had more than doubled and, 6 months after, more than tripled. After this the rate of gain was much slower. The table at the top of the next page shows the weights during a 17-month period.

This rapid increase in weight was associated with a marked polyphagia. The monkey was observed to eat constantly and showed great greediness. His

[1] Aided by a grant from the Rockefeller Foundation.

Date	*Weight in kg.*	*Date*	*Weight in kg.*
5/28/35 (Operated)	2.6	11/ 4/35	7.5
6/20/35	3.6	1/ 3/36	8.2
7/ 3/35	4.2	2/13/36 (Sick for 5 days)	8.1
7/13/35	4.5	3/14/36	8.7
7/25/35	5.4	5/28/36	8.9
8/17/35	6.3	10/29/36	9.07
9/17/35	6.6	11/11/36 (Killed)	

food pouches were frequently stuffed with food, giving the appearance of a large goiter. The adiposity was largely confined to the neck and trunk, the abdomen showing a tremendous protrusion (fig. 1).

Diabetes mellitus. Unfortunately, the time of onset of diabetes mellitus in this animal was not determined. It was observed that the monkey drank large amounts of water but this was at first attributed to the large food intake. A little over 9 months after the operation the animal was put in a metabolism cage, because of a suspicion that it might have developed diabetes insipidus. Because of the high specific gravity of the large volume of urine a Benedict's qualitative test was applied and revealed the presence of a marked glycosuria. A week later the blood sugar was found to be 192.2 mg. in 100 cc. One year after the operation the blood sugar was 230.8 mg. per cent, and essentially the same concentration was found 3 months later. The response to insulin was determined 17 months after the operation, the fasting blood sugar falling from 257.6 mg. per cent to 179 mg. per cent after 1 hour. The dose of insulin was 10 U and the body weight was 9.07 kg.

There was marked polyuria and polydipsia at this stage in the course of the disease. During a 24-hour period of fasting, there was excreted 800 cc. of urine with a specific gravity of 1.036 and a concentration of 8.6 per cent glucose. The urine output for 24 hours contained 69.4 gm. of sugar. Acetone was present in large amounts.

In spite of the fact that no attempt was made to control the diabetes either by diet or insulin, the monkey continued to remain in good health.

Development of the testes. Genital atrophy did not occur in this monkey. When the animal was killed, viable spermatozoa were observed microscopically in a saline suspension of testicular tissue. There is evidence that the testes descended prematurely. According to Hartman and Strauss (1), the permanent descent of the testes does not occur in the Rhesus monkey until the middle of the 6th year, but temporary descent with subsequent return to the inguinal canal may occur earlier. From the weight and crown-rump length of our monkey, we calculated its age to be between 24 and 30 months at the time of operation. The testes were first found to have descended 4 months after the operation, or at an estimated age of about 28 to 34 months, and they were not again observed to leave the scrotum. Our monkey was killed a little more than 17 months after operation, or at an estimated age of between 41 and 47 months. However, his crown-rump length at this time was 47 cm., which is greater than Hartman and Strauss's specimen, 60 months old. There would appear, therefore, to have been an acceleration of growth during these 17 months, as well as a premature descent of the testes. But there can be no certainty as to this, in the absence of information in regard to the normal variability of growth and development in the monkey.

Temperature regulation. This monkey showed some impairment in ability to prevent chilling or overheating. In this respect it resembled others of the same series, which showed no obvious metabolic disturbances (2).

ANATOMICAL FINDINGS

On November 11, 1936 the monkey was killed by bleeding, and carefully autopsied. There was a heavy layer of fat over the abdomen which measured from 2 to 2.2 cm. in thickness. No ascites or pleural effusion was present. The omentum and mesentery were heavily infiltrated with fat. The

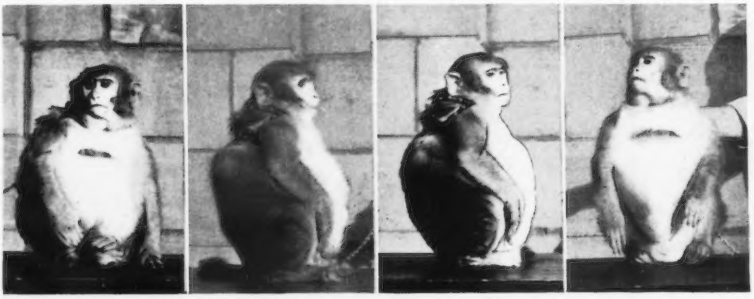

Fig. 1. Photographs of *M36* showing adiposity of the neck and trunk, taken 163 days after the placing of lesions in the hypothalamus.

fatty layer over the thorax was likewise heavy and in the groin there was a layer of fat 7 mm. in thickness. On the extremities the fatty development was much less marked, but the axillary cavity was full of fat and there was a good deal around the neck. The heart, kidney and adrenals were embedded in masses of adipose tissue.

Pieces of the thyroid, kidney, pancreas, spleen, adrenals, testes, liver and parathyroids were removed and fixed in Zenker-formol. The brain was removed with the hypophysis attached. The latter was cleanly separated from the hypothalamus by a section through the infundibular stem, and fixed in Zenker-formol. The region of the hypothalamus was cut away and fixed in formaldehyde U.S.P. (1:10). The organs were embedded in paraffin and sections cut 10 μ in thickness and stained with haematoxylin and eosin. The hypothalamus was embedded in celloidin and 50 μ serial sections cut and stained alternately with cresyl violet and the Weil method. The hypophysis was embedded in paraffin, cut serially at 5 μ, mounted so that alternate rows were on adjacent slides, which were then stained alternately by the eosin-azure II method and by a technic which was suggested to us by Dr. H. O. Haterius and which is based on the method of Severinghaus.

The organs and the hypophyses of 2 immature unoperated male monkeys with crown-rump lengths 41 and 42 cm. were similarly treated and used as microscopic controls.

Lesions in the hypothalamus. On each side the 3 lesions had fused into a single elongated one, extending backward through the hypothalamus from in front of the infundibulum to the level of the rostral border of the mam-

millary body. These long lesions were not symmetrically placed. On the right side the medial part of the hypothalamus and wall of the third ventricle were involved, on the left the lesion was in the lateral part of the hypothalamus, damaging the medial edge of the internal capsule and basis pedunculi.

The supra-optic and filiform nuclei were normal in size, but there was perhaps some slight decrease in the number of cells. The ventromedial hypothalamic nucleus was largely destroyed on one side, and on both sides the lateral hypothalamic nucleus and the nucleus of the ansa lenticularis were

Fig. 2. Photomicrograph of a section through the hypothalamus showing two large bundles of myelinated fibers entering the median eminence. *ME* = median eminence. *OT* = optic tract.

damaged. The fornix was completely interrupted on both sides but the mammillothalamic tract was intact.

The lesions in the hypothalamus were very similar to those in a number of other monkeys which showed no tendency to adiposity, and we see no reason to attribute the syndrome shown by this animal to these lesions. In a series of 28 brains from monkeys which had been studied with special reference to disturbances in heat regulation the amount of damage to each of the hypothalamic nuclei was carefully determined and tabulated. In this series each nucleus was damaged or destroyed in at least two of the brains. No single nucleus escaped injury throughout the entire series. Since none of these 28 monkeys showed adiposity, it can be said that there is no nucleus in the hypothalamus whose complete integrity is essential for proper regulation of fat metabolism.

Figure 2 shows the only striking peculiarity of this brain. Two rather large bundles of myelinated fibers can be seen running ventrally into the median eminence (the attachment of the infundibulum to the hypothalamus). From the gray matter near the dorsomedial edge of the optic tract the fibers converge on either side into a compact bundle as they enter the median eminence. A few sections farther rostrally the bundles are seen running

downward just behind the optic chiasma. Here they are closely related to Meynert's commissure, and the appearance is given as if they formed a part of this commissure. Their origin cannot be accurately determined. The possibility that they may be fibers of the hypothalamico-hypophyseal tract which have acquired myelin sheaths must not be overlooked. Probably a majority of the fibers pass down the infundibular stem but as to this point it is not possible to be certain because the stem was cut away from the median eminence.

Myelinated fibers do not enter the median eminence in the 2 normal monkey brains of which we have serial frontal sections, but a few such fibers have been seen in a number of the monkeys with hypothalamic lesions, which did not become obese. Whether such fibers occur as a normal variation or whether they are in any way related to the lesions remains uncertain. In none of the other operated monkeys were these fibers anywhere near so numerous as in the monkey under consideration.

The hypophyses of the obese monkey (*M36*) and of the 2 controls (*M11* and *12*), which had been cut, and the sections mounted so that alternate rows of sections were stained by the Haterius method and by eosin and azure II, were studied with reference to their cell content. Those prepared by the Haterius method showed the best differentiation of the basophils, although the stain was not uniform or sharply differential.

Dr. A. T. Rasmussen very kindly examined the sections of the hypophyses of the 3 monkeys, and made as accurate a count of the basophils as was possible in view of the unsatisfactory character of the preparations. In his report he states:

"Every third microscopic field in every third row of fields in one section from the middle of each row of sections covered by B. & L., 1.9 mm. fluorite system oil-immersion objective and No. 10 ocular, and strong unfiltered artificial light. Many basophils had to be identified by their large Golgi apparatus and granular cytoplasm (when the stain was too pale to color the granules blue) and occasionally by their vacuoles. The figures below are not trustworthy as to detail but should give the general (relative) number of basophils per unit volume of anterior lobe tissue.

Animal	No. of Sections	No. of Fields	No. of Basophils	Average No. of Basophils per Field
M11	10	188	2836	15
M12	17	279	1113	4
M11 + *M12*	27	467	3949	8.5
M36	13	430	7443	17.3

"The two normal animals varied enormously. How much of this is due to not having similarly located sections I cannot say. But it can hardly be denied that *M36* had absolutely and relatively more basophils than the other two. In *M36* the basophils also appeared more hyperplastic (cell arrangement). In *M12* the acidophils were better stained and appeared particularly numerous but mostly small in size."

The neural lobe appeared normal, although there was probably a slight amount of hypercellularity which can be correlated with partial interruption of the hypothalamico-hypophyseal tract, especially the tubero-hypophyseal component.

Other organs. The islets of Langerhans in the pancreas showed varying degrees of hydropic degeneration, which appeared to be of the same type as that described by Allen (3). The vacuoles varied in size. In some of the cells the cytoplasm was largely replaced by fluid. The exocrine portion of the pancreas seemed to be normal.

The testes were mature and normal. Cells in various stages of spermatogenesis were present and the lumina of the seminiferous tubules were full of mature spermatozoa. The thyroid appeared normal and there was no evidence of hyperplasia or hypoplasia. The adrenal gland showed no obvious abnormalities in either the cortex or the medulla. The gland was larger than those obtained from the 2 control monkeys but no larger than some we have taken from other normal monkeys. The liver, kidney and spleen were normal. The parathyroids showed some indications of hyperplasia.

COMMENT

The interpretation of the clinical and pathological findings in this monkey are beset with many difficulties. Four possible explanations may be suggested.

First, the syndrome may have been caused by the hypothalamic lesions. In favor of this is the fact that the adiposity began to develop immediately after operation. However, the lesions were similar to those which have been produced in other monkeys and in cats which failed to develop adiposity or diabetes mellitus.

Second, some hypophyseal disturbance may have been responsible for the syndrome. The most unusual anatomical finding was the presence of two large bundles of myelinated fibers in the median eminence, which probably made their way down the hypophyseal stem. But how these fibers could have been responsible for the adiposity is not clear. It is of interest to note that the simultaneous occurrence of adiposity and diabetes mellitus is to be found in Cushing's disease (4). We were, therefore, concerned with the cellular picture of the anterior hypophysis. No basophilic adenoma was present. Furthermore, we do not believe that the increase in basophils was sufficiently marked to indicate a significant hyperplasia. Nevertheless, it is worth noting that our monkey did present some of the elements of the syndrome of basophilic adenoma. The marked girdle adiposity and the hyperglycemia and glyclosuria have already been stressed. A number of investigators have pointed out that when Cushing's disease occurs in children there is a premature development of the gonads (5). There was some indication that in this monkey premature descent of the testes occurred.

Third, a disturbance in carbohydrate metabolism may have been the primary factor, the adiposity developing secondarily. Unfortunately the precise time of onset of the diabetes was not determined.

Fourth, involvement of the hypothalamico-hypophyseal system may have been the primary factor (6). We do not believe that the evidence warrants such a conclusion. We have seen many animals with all degrees of degeneration of this system but have never observed adiposity or diabetes mellitus. In the monkey under consideration the supraoptic component of the hypo-

thalamico-hypophyseal system was practically intact, the tubero-hypophyseal component showing more extensive involvement.

SUMMARY

An account is given of a monkey which developed adiposity within 3 weeks after lesions had been placed in the hypothalamus. At autopsy normal mature testes were found, and there was some evidence that premature descent of the testes may have occurred. An associated diabetes mellitus was explained by the finding in the pancreas of hydropic degeneration of the isles of Langerhans. The lesions in the hypothalamus and the histology of the hypophysis, suprarenal glands and thyroid were studied without yielding clear evidence concerning the cause of the adiposity.

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MISCELLANEOUS EXPERIMENTS ON THE ESTROGEN-PROGESTERONE INDUCTION OF HEAT IN THE SPAYED GUINEA-PIG[1]

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Heat can be induced in a certain percentage of spayed female guinea-pigs by the injection of an estrogen alone, but the injection of an estrogen followed by progesterone is more effective (1, 2). This observation directed attention to several supplementary problems. Among these were, *a)*, differences between estrogen-induced heat and estrogen-progesterone-induced heat, *b)*, the frequency with which spayed females can be brought into heat by estrogen-progesterone administration, and *c)*, the response to estrogen-progesterone treatment given by spayed animals in which heat was short or failed to occur prior to ovariectomy.

Investigation of these subjects required the use of different groups of animals and methods, which are described with the results.[2]

EXPERIMENTAL

Differences between estrogen-induced heat and estrogen-progesterone-induced heat. Certain differences were apparent during the earlier experiments. Even when large doses of estrogens were injected, the percentage of animals which came into heat was less than when small quantities of estrogen were followed by progesterone. A similar result was obtained from recent experiments in which the quantity of estrogen used for the estrogen-induced heat and the estrogen-progesterone-induced heat was the same. Following 138 injections of 200 I.U. of theelin[3] into 81 animals, heat occurred only 14 times or following only 10.1 per cent of the injections. On the other hand, when progesterone[4] was injected into the same animals on the 24th, 30th or 48th hour after the estrogen injection, heat occurred following 80 to 86.6 per cent of the injections (2).

It was also found during the earlier work that the latent period (interval between injection and the beginning of heat) following estrogen administration averaged 34.7 hours. On the other hand, once an animal was conditioned by estrogen treatment, the latent period following progesterone injection averaged only 5.6 hours (1, 2).

[1] This investigation was supported by a grant from the Committee for Research in Problems of Sex, National Research Council.
[2] We acknowledge with thanks the supply of oil-soluble theelin provided by Dr. Oliver Kamm of Parke, Davis and Company, and the generous contributions of progynon-B and proluton made by Dr. Erwin Schwenk of the Schering Corporation.
[3] Theelin, Parke, Davis & Company.
[4] Proluton, Schering Corporation.

The more recent investigations have revealed additional differences. The beginning of the estrogen-progesterone-induced heat is well defined, resembling in this respect the beginning of heat in the normal animal (3). The beginning of the estrogen-induced heat, on the other hand, is less well defined and cannot be determined with the same certainty.

When similar quantities of estrogen were injected the estrogen-progesterone-induced heat frequently was shorter than the estrogen-induced heat. Following 78 injections of 200 I.U. of theelin and 0.2 I.U. progesterone, the length of heat averaged 7.2 hr., range 3 to 11 hr. (2). Following the

TABLE 1. LENGTH OF THE LATENT PERIODS AND LENGTH OF HEAT IN ANIMALS BROUGHT INTO HEAT FIRST WITH ESTROGENS AND SUBSEQUENTLY WITH PROGESTERONE

Animal	Estrogen induced heat		Time in hr. between the end of the estrin-induced heat and the progesterone injection	Estrogen-progesterone induced heat	
	Latent period in hr.	Length of heat in hr.		Latent period in hr.	Length of heat in hr.
974	<45	not known	<15	4.5	6.5
988	<45	not known	<15	4.5	6.0
999	<36	not known	<60	5.0	8.5
1586	<57	not known	< 3	3.0	12.5
1681	<48	not known	<12	5.0	10.5
4y	35.0	12.0	1.0	4.0	7.0
6y	44.5	13.5	1.0	5.0	9.0
9y	<38	>5	66.0	5.0	9.0
9y	35.5	8.0	29.0	6.5	3.5
10y	38.0	8.5	14.0	5.0	9.0
10y	34.5	10.0	28.0	3.5	11.0
10y	35.0	8.5	1.0	4.5	8.0
19y	35.5	8.0	29.0	6.0	8.5
40y	34.5	12.0	26.0	4.5	11.0
40y	35.0	8.5	1.0	5.5	7.0
Average (when exact length is known)	36.3	9.9		4.7	8.0

injection of 200 I.U. of theelin alone, the length of heat in the 9 instances in which it was known averaged 9.9 hr., range 8 to 13.5 hr. (table 1).

The responsiveness of animals to progesterone injections following estrogen-induced heat differs from that following estrogen-progesterone-induced heat. During the early part of the work experiments bearing on this point were performed on only 4 animals. Recently, a more extensive study has been made.

Two procedures were used. In the first, animals were observed for heat after the injection of an estrogen alone. Heat occurred following 15 of 180 injections (138 injections of 200 I.U. of theelin, 14 of which induced heat, and 42 injections of 50 I.U. of progynon-B, 1 of which induced heat). One to 66 hours after the end of the estrogen-induced heat a single injection of 0.2 I.U. of progesterone was made. In all cases a second heat occurred, and without exception the length of the latent periods and heat were similar to those that ordinarily follow estrogen-progesterone treatment (table 1).

The second procedure involved the use of the 41 animals which did not come into heat after the injection of 50 I.U. of progynon-B until they were injected 48 hours later with progesterone. The animals conditioned with progynon-B were used because they were to be injected again with progesterone 48 hours after the first injection of this hormone. It was therefore important to use an estrogen which would keep most animals conditioned 96 hours or longer (2).

The 41 animals were divided into 2 groups. The first group of 20 was injected a second time with 0.1 I.U. of progesterone 1 hour after the end of the heat period induced by the first injection. Of these, 1 displayed a heat period of 5 hours following a latent period of 5 hr. Thirty-three to 37 hr. after the second injection of progesterone, therefore 96 hr. after the condition-

TABLE 2. HEAT RECORDS OF ANIMALS INJECTED WITH ESTROGEN-PROGESTERONE AT 3-DAY INTERVALS

Animal		1st inj.	2nd inj.	3rd inj.	4th inj.	5th inj.
		hours	*hours*	*hours*	*hours*	*hours*
1737	Latent period	4.0	3.0	4.0	3.5	∞
	Length of heat	10.5	14.0	8.0	5.0	0
1797	Latent period	3.5	5.0	4.0	3.5	3.5
	Length of heat	11.0	4.0	7.5	8.0	8.5
1823	Latent period	3.5	∞	7.5	∞	∞
	Length of heat	10.0	0	4.0	0	0
1827	Latent period	4.5	3.5	4.5	∞	4.0
	Length of heat	5.0	5.0	5.5	0	7.0
1862	Latent period	4.0	3.0	3.0	3.5	3.5
	Length of heat	10.5	8.0	8.5	9.0	8.5
1932	Latent period	3.5	3.5	9.0	∞	3.5
	Length of heat	8.0	5.0	2.5	0	9.0
Average	Latent period	3.8	3.6	5.3	3.5	3.6
	Length of heat	9.2	7.2	6.0	7.3	8.3

ing injection of progynon-B, the 20 animals were given a third injection of 0.1 I.U. of progesterone. This time no animals came into heat.

The second group, composed of 21 animals, was given a second injection of 0.1 I.U. of progesterone 31 to 38 hr. after the end of the heat induced by the first injection, and therefore 96 hr. after the conditioning injection of progynon-B. In no case was heat induced.

As stated above, most animals injected with 50 I.U. of progynon-B remain conditioned longer than 96 hr. Consequently, the failure of all but 1 of the experimental animals to come into heat following 61 instances of second and third injections of progesterone must be attributable either to the failure of the animals to remain conditioned or to become reconditioned after the first progesterone injection.

The experiments demonstrate that in general a second progesterone injection any time from 1 to 38 hr. after the end of a progesterone-induced heat is ineffective in producing a second heat, whereas injection of this hormone

any time from 1 to 66 hr. after the estrogen-induced heat is effective. The first part of the conclusion confirms the earlier experiments bearing on the same point (1). The latter part corrects the statement that a second heat cannot be induced after an estrogen heat unless a second conditioning injection of estrogen is made.

Smith and Smith (4) and Pincus and Zahl (5) have shown that, following the injection of an estrogen into ovariectomized rabbits, a greater percentage of the estrogen or its active conversion products can be recovered from the urine if progesterone is injected simultaneously with, or shortly after, the estrogen. If this is found to be true in the spayed guinea-pig, it may account for the failure to produce more than one progesterone-induced heat following a single injection of estrogen. Furthermore, it will be shown in the next section that the injections of progesterone at short intervals are effective in inducing heat, provided intermediate injections of an estrogen are given.

The frequency with which spayed guinea-pigs can be brought into heat by estrogen-progesterone administration. Each of 6 mature spayed females, after being injected with 100 I.U. of progynon-B followed 48 hr. later with 0.2 I.U. of progesterone, was observed for heat. The procedure was repeated, beginning on the 3rd, 6th, 9th, and 12th days after the initial progynon-B injections. Consequently, each animal was given estrogen-progesterone injections 5 times in 15 days. This experimental period was chosen because its length approximates that of the normal cycle during which heat occurs only once.

The results are summarized in table 2. The latent periods and length of heat were normal. Two animals were brought into heat 5 times, three 4 times and 1 only twice. The experiment demonstrates that a spayed guinea-pig can be brought into heat repeatedly by estrogen-progesterone injections made at short intervals.

The response of estrogen-progesterone treatment given by spayed animals in which heat was short or failed to occur prior to ovariectomy. Four control and 9 experimental animals were used. The control animals were spayed after being selected at random from animals displaying normal heat periods. The experimental animals were those which showed the greatest tendency to heat failure in a colony of more than 300 individuals.

Three of the experimental animals were observed continuously, day and night, between October 10, 1935 and March 31, 1936 and again between September 10 and November 17, 1936 when ovariectomy was performed. In the 3 animals 1 heat period was as long as 9 hr., but the other 14 were shorter than normal, and 13 cases of heat failure were recorded. Two of the 3 animals did not come into heat during the second period of observations.

Two experimental animals were observed until they were 138 and approximately 180 days old, respectively, by which time heat had not occurred. One entire ovary and all but a fragment of the other were then removed. After the operation they were observed continuously during a 50-day period, and 5 months later during a 67-day period. Heat had not occurred by the end of this time and the remaining ovarian fragments were removed.

Four animals which failed to come into heat were observed over a period of 67 days, when they were approximately 90 to 160 days of age. They were spayed when the observations were terminated.

After ovariectomy both experimental and control animals were injected

TABLE 3. HEAT RECORDS OF ESTROGEN-PROGESTERONE-INJECTED SPAYED ANIMALS IN WHICH HEAT WAS SHORT, OR HAD FAILED TO OCCUR PRIOR TO OVARIECTOMY

	Animal	Inj.	Latent period in hr.	Length of heat in hr.
Experimental group	*955*	First Second Third	∞ ∞ ∞	0 0 0
	1135	First Second Third	∞ ∞ ∞	0 0 0
	1098	First Second	∞ 9.0	0 1.5
	1312	First Second	∞ 6.0	0 5.5
	1390	First Second	∞ 6.5	0 4.0
	1788	First Second	∞ 8.0	0 2.5
	1852	First	11.5	1.5
	AJ	First	9.0	4.5
	XHA	First	8.0	4.0
	Average (when heat occurred)		8.3	3.8
Control group	*1234*	First	4.5	5.5
	1786	First Second	3.0 3.5	10.0 8.5
	1789	First Second	3.0 3.5	10.0 9.0
	1971	First Second	3.5 3.5	9.5 9.0
	Average		3.5	8.8

from 1 to 3 times each with 100 I.U. of progynon-B followed 48 hours later with 0.2 I.U. of progesterone. When more than 1 series of injections was made, the interval from one to another was 9 to 12 days. The results are summarized in table 3.

After each series of injections into the control animals, heat occurred, its length and the length of the latent period being similar to those that ordinarily follow estrogen-progesterone treatment. Of the experimental animals, 2 did not respond to any one of the 3 injections, 4 came into heat only

after the second injection, and 3 were brought into heat by the first injection. However, when heat was induced, the latent period was always long and heat short, compared with the controls. In the 8 animals whose ovaries were studied, the condition was normal and ovulation was occurring (6). Therefore the assumption is that these animals were capable of displaying heat, but that the threshold for estrogen-progesterone action was high and that they would respond only to the action of larger than normal quantities of estrogen followed by progesterone.

SUMMARY

Estrogen-induced heat and estrogen-progesterone-induced heat differ in several respects. The beginning of an estrogen-induced heat is less well defined. The length of the estrogen-induced heat in the animals studied was frequently longer. The injection of progesterone after the end of an estrogen-induced heat is followed by a second heat period, whereas an injection following an estrogen-progesterone-induced heat is without effect. Adult spayed guinea-pigs can be brought into heat as often as 5 times within a period that approximates the length of a normal cycle. Animals in which the ovarian condition was normal and in which ovulation was occurring, but in which heat was short or did not occur prior to ovariectomy, were brought into heat when larger than normal quantities of estrogen followed by progesterone were injected. However, when heat was induced by this treatment the latent period was long and heat short. It is assumed that in these animals the short heat periods and the failure of heat were associated with a low sensitivity to hormonal action.

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QUANTITATIVE STUDIES OF EXPERIMENTALLY INDUCED SEXUAL RECEPTIVITY IN THE SPAYED GUINEA-PIG[1]

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In 1935, Young, Dempsey and Myers (1) reported studies of the copulatory response in the normal adult female guinea-pig which can be elicited at the time of heat by stroking the animal in the dorso-lumbar region. Later Dempsey, Hertz and Young (2) demonstrated the synergistic action of estrogen followed by progesterone in inducing this behavior in the spayed female guinea-pig. Simultaneous injections had no effect and it became apparent that the estrogen must act for a period of time before the reaction can be completed by progesterone. The time of action of the estrogen prior to the injection of progesterone is called the conditioning[2] period; the interval between the administration of progesterone and first copulatory response is the latent period.

It seemed important to analyze quantatively the factors of the estrogen-progesterone reaction by determining, *a)*, the conditioning period after which the greatest number of animals is brought into heat, *b)*, the length of time an animal remains conditioned following a single injection of estrogen, *c)*, the length of the latent period and heat, *d)*, the effect of different estrogens, *e)*, the effect of different quantities of estrogen and progesterone. The value of such an analysis lies in its contribution to an understanding of the physiology of the conditioning process by supplying information on the time factors involved in the absorption, inactivation and excretion of the hormones responsible for heat. The analysis also furnishes a method for the accurate bio-assay of estrogens and progesterone.

PROCEDURE AND RESULTS

Two colonies of guinea-pigs were used. Eighty mature females comprising the 1st colony, were spayed and injected during the spring and summer of 1937; 50 animals, comprising the 2nd colony, were spayed and injected during the following fall and winter. With the use of 2 different groups of animals a check was available on the results, which proved to be reproducible. How-

[1] This investigation was supported by a grant from the Committee for Research in Problems of Sex, National Research Council.

[2] The choice of the word 'condition' to describe the action of estrogens in preparing an animal for heat may have been unfortunate because so many associate the Pavlovian usage with it. The words 'prepare,' 'prime' and 'sensitize' have been suggested, but equally serious objections can be raised to each. The selection of a completely satisfactory descriptive term cannot be made until the nature of the estrogen action in the induction of heat is known.

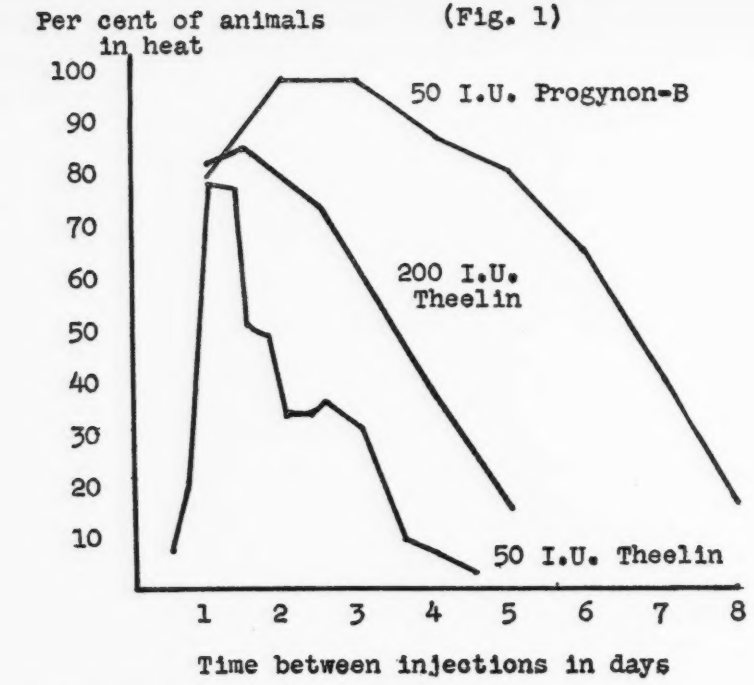
ever, when animals were injected for the first time they were found to be quite refractive. Thus, only about 52 per cent of the animals gave the copulatory response when injected with 50 I.U. of theelin followed by 0.2 I.U. of progesterone, whereas, following the second treatment 80 per cent gave the response. The first treatment has been designated as 'priming' the animals. A somewhat comparable situation exists in connection with the production of menstruation in immature monkeys. When such monkeys are first treated with estrogen they require several times the menstrual dose for adults. If, however, one half the threshold for a first menstruation is given after a short interval, menstruation follows (3).

After priming, the guinea-pigs were submitted to 6 procedures of injections. The hormones were dissolved in corn oil and injected subcutaneously in the axillary region. The end-point of the hormonal action was sexual receptivity. After each series of injections the animals were given a two-weeks' rest period to insure complete removal of any remaining hormons. That such a rest period is adequate is indicated by closure of the vaginal membrane which usually occurred within 8 days after the estrogenic treatment employed. However, following injections of progynon-B, which exhibits prolonged activity, a three-weeks' rest period was deemed advisable. The following procedures were used.

A). Animals were injected with 50 I.U. of theelin and grouped according to the time 0.2 I.U. of progesterone was injected.[3] The interval between injections was varied from 12 to 108 hr., and the results are summarized in table 1. When the percentage of animals that came into heat is plotted against the conditioning time, a frequency distribution curve is obtained (fig. 1). The percentage of animals in heat rose from 7 per cent at 12 hr. to a maximum of 79 per cent at 24 hr. and then declined to 6 per cent at 108 hr. When the latent periods and lengths of heat are plotted against the conditioning time (fig. 2), they show, *a),* a horizontal trend and, *b),* an inverse relation to each other: as length of heat increases, latent period decreases and vice versa. To determine statistically if the latter is significant, the coefficient of correlation between latent period and length of heat was calculated and found to be —.66 indicating the close inverse relation. The average length of the combined latent period and length of heat for all groups is 11.82 hr. (The coefficient of correlation was calculated by the product-moment method. The P.E. of the mean latent period is 0.31 hr., the P.E. of the mean length of heat is 0.12 hr.)

B). The animals were injected with 200 I.U. of theelin and grouped according to the time 0.2 I.U. of progesterone was injected. The interval between injections was varied from 24 to 120 hr. and the results are summarized in table 2. As before, plotting percentage of animals coming into heat against conditioning time produces a frequency curve (fig. 1) with a maximum of 90 per cent at 30 hr. which decreases to 14 per cent at 120 hr. The latent periods and lengths of heat are but slightly different from results

[3] Appreciation is expressed for the generous contributions of theelin by Dr. Oliver Kamm of Parke, Davis and Company, and for proluton (progesterone) supplied through the courtesy of Dr. Erwin Schwenk by the Schering Corporation.



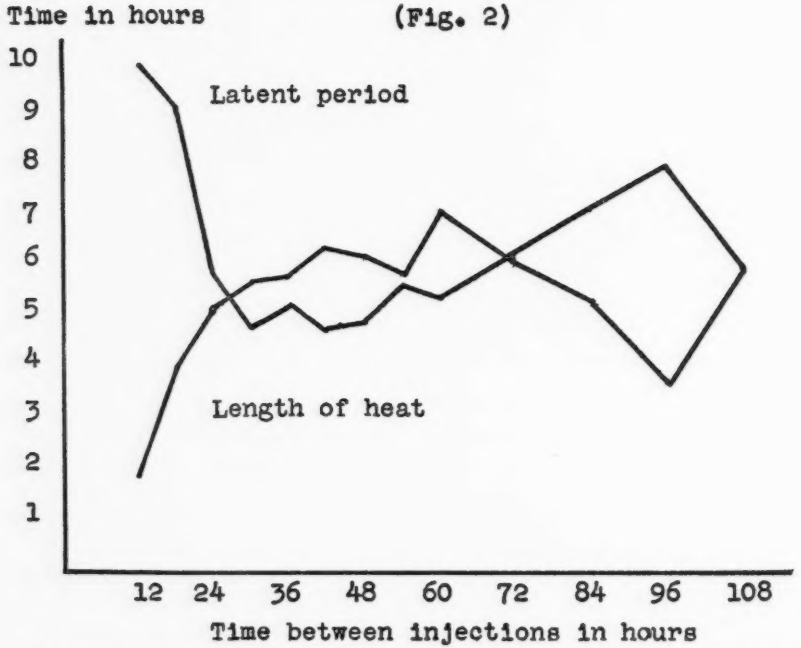


Fig. 1. Comparison of percentage of spayed animals brought into heat by different procedures of estrogenic conditioning. Fig. 2. Variation in latent period and length of heat in spayed guinea-pigs brought into heat by 50 I.U. of theelin and 0.2 I.U. of proluton as time between injections is varied.

TABLE 1. EFFECT OF A SINGLE INJECTION OF 50 I.U. OF THEELIN FOLLOWED BY A SINGLE INJECTION OF 0.2 I.U. OF PROLUTON IN CAUSING HEAT IN SPAYED GUINEA-PIGS

Time between injections	Number injected	Number in heat	% in heat	Mean latent period	Mean length of heat	Combined latent and length
Hours				Hours	Hours	Hours
12	13	1	7.7	10.00	2.00	12.00
18	15	3	20.0	9.16	4.00	13.16
24	39	31	79.5	5.97	5.22	11.19
30	28	22	78.5	4.92	5.69	10.61
36	30	16	53.3	5.38	5.79	11.17
42	30	15	50.0	4.84	6.41	11.25
48	43	15	34.9	4.96	6.32	11.28
54	43	15	34.9	5.56	5.86	11.42
60	32	12	37.5	5.42	7.10	12.52
72	32	11	34.3	6.25	6.10	12.35
84	34	3	9.1	7.16	5.33	12.49
96	28	2	6.9	8.50	3.75	12.25
108	30	2	6.6	6.00	6.00	12.00

in *A)*, though there was a fourfold increase in the theelin injected. When plotted against conditioning time (fig. 3) they show a horizontal trend and an inverse relationship. The combined latent period and length of heat averages 12.32 hr. for the series.

C). In order to compare the above results with those obtained using another estrogen, the animals were injected with 50 I.U. of dihydroxyestrin-benzoate (progynon-B, Schering) and grouped according to the time 0.2 I.U. of progesterone was injected. The results are summarized in table 3 and represented graphically in figures 1 and 4. Heat occurrence extended to the 8th day. This is not surprising because the dihydro compounds and the esters of estrin exhibit prolonged and persistent activity. Latent periods and lengths of heat show definite horizontal trends and are inversely related. Their sum approximates 12.69 hr.

D). The effect of varying the amount of progesterone was determined 24 hr. after conditioning the animals with 50 I.U. of theelin. The results are shown in table 4. In figure 5 dosages of progesterone are plotted against the percentage of animals giving the heat response; the curve is sigmoid.

E). The amount of theelin injected was varied in different groups of animals and 24 hr. later 0.2 I.U. of progesterone was injected (table 5). The

TABLE 2. EFFECT OF A SINGLE INJECTION OF 200 I.U. OF THEELIN FOLLOWED BY A SINGLE INJECTION OF 0.2 I.U. OF PROLUTON IN CAUSING HEAT IN SPAYED GUINEA-PIGS

Time between injections	Number injected	Number in heat	% in heat	Mean latent period	Mean length of heat	Combined latent and length
Hours				Hours	Hours	Hours
24	13	11	84.6	4.68	8.90	13.58
30	15	13	86.6	4.73	8.65	13.38
48	10	8	80.0	3.70	7.56	11.26
60	16	12	75.0	5.28	7.04	12.32
96	47	17	36.1	5.78	6.97	12.75
108	35	10	28.5	5.60	5.95	11.55
120	49	7	14.3	5.86	5.57	11.43

TABLE 3. EFFECT OF A SINGLE INJECTION OF 50 I.U. OF PROGYNON-B FOLLOWED BY A SINGLE INJECTION OF 0.2 I.U. OF PROLUTON IN CAUSING HEAT IN SPAYED GUINEA-PIGS

Time between injections	Number injected	Number in heat	% in heat	Mean latent period	Mean length of heat	Combined latent and length
Days				Hours	Hours	Hours
1	10	8	80.0	5.10	7.93	13.03
2	10	10	100.0	4.25	6.85	11.10
3	9	9	100.0	5.10	7.05	12.15
4	9	8	88.0	4.87	7.60	12.47
5	12	10	83.3	5.60	6.80	12.40
6	12	8	66.6	6.00	8.00	14.00
7	12	5	41.6	5.20	8.00	13.20
8	12	2	16.6	5.00	8.20	13.20

theelin dosages plotted against the percentage of animals brought into heat gives a sigmoid curve (fig. 6).

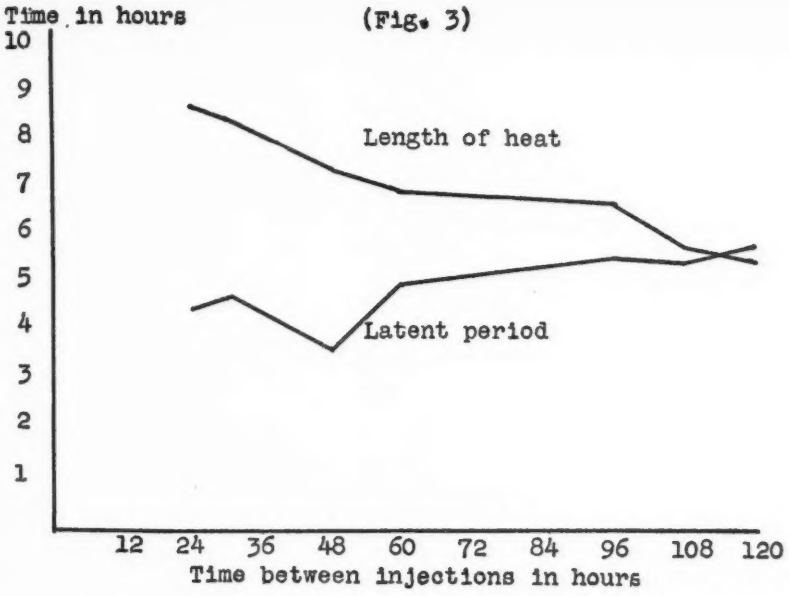
F). It was desired to know the effect of divided estrogenic dosage on the heat response and the effect of estrogen made readily available to the organism as after an intravenous injection. Animals were given a single injection of 12.5 I.U. of theelin on the 0, 12th, 24th and 36th hr. On the 48th hr. 0.2 I.U. of progesterone was administered to 1 group and 0.2 I.U. of progesterone plus 50 R.U. of water-soluble theelin given intravenously to the other. In the 1st group the latent period was shorter and heat longer than when single injections of 50 I.U. of theelin were administered. However, the sum of these two, 12.0 hr., shows little change from results of single injections (table 6). When the intravenous theelin was given there was also a significant shortening of the latent period but the length of heat was much greater. Thus, the effect of theelin made readily available is to increase the length of heat to 15.7 hr. as compared to 9.30 hr. for animals in the first group.

DISCUSSION

Before the experimental work was begun, it was thought that as the conditioning period was lengthened there would be significant changes in either length of latent period or heat, or both. In the animals conditioned with 50 I.U. of theelin, and 50 I.U. of progynon-B, no such changes occurred. In the animals conditioned with 200 I.U. of theelin, heat tended to become shorter and latent period longer as the length of the conditioning period was increased. However, the changes that occurred were not comparable with the

TABLE 4. EFFECT OF VARIATIONS IN AMOUNT OF PROLUTON INJECTED 24 HOURS AFTER A SINGLE INJECTION OF 50 I.U. OF THEELIN IN CAUSING HEAT IN SPAYED GUINEA-PIGS

Amount of proluton	Number injected	Number in heat	% in heat	Mean latent period	Mean length of heat	Combined latent and length
				Hours	Hours	Hours
0.4 I.U.	12	11	91.7	5.00	5.64	10.64
0.2 I.U.	39	31	79.5	5.97	5.22	11.19
0.1 I.U.	15	11	73.3	4.50	6.59	11.09
0.05 I.U.	15	3	20.0	5.16	3.83	8.99
0.025 I.U.	16	2	12.5	4.25	6.00	10.25



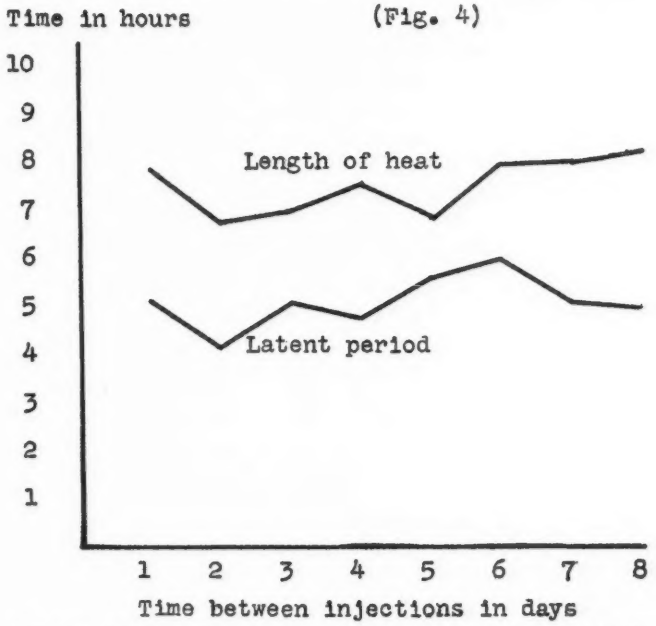


Fig. 3. Variations in latent period and length of heat in spayed guinea-pigs brought into heat by 200 I.U. of theelin and 0.2 I.U. of proluton as time between injections is varied. Fig. 4. Variations in latent period and length of heat in spayed guinea-pigs brought into heat by 50 I.U. of progynon-B and 0.2 I.U. of proluton as time between injections is varied.

TABLE 5. EFFECT OF VARYING QUANTITIES OF THEELIN INJECTED INTO SPAYED GUINEA-PIGS FOLLOWED 24 HR. LATER BY AN INJECTION OF 0.2 I.U. OF PROLUTON IN CAUSING HEAT

Amount of theelin	Number injected	Number in heat	% in heat	Mean latent period	Mean length of heat	Combined latent and length
				Hours	Hours	Hours
200 I.U.	13	11	84.6	4.68	8.90	13.58
50 I.U.	39	31	79.5	5.97	5.22	11.19
40 I.U.	10	6	60.0	5.50	5.41	10.91
30 I.U.	17	9	52.9	5.33	8.05	13.38
20 I.U.	15	5	33.3	5.00	8.30	13.30

decrease in percentage of animals brought into heat after long periods of conditioning. Consequently, for a given procedure, animals that come into heat do so after a fairly definite latent period and have a rather definite length of heat, while the sum of the latent period and length of heat approximates a constant. Because of this, an 'all-or-none' mechanism of receptivity is postulate. Thus, if the hormonal stimulation is adequate to induce heat, the animal reacts completely or not at all. It should be emphasized that length of heat in individual animals appears to depend on the sensitivity of that animal (4). In this study some animals were consistent in showing short latent periods and long heat and other animals the reverse. This difference in sensitivity may account for variations in heat length and latent periods that were observed.

It is also interesting that, despite quantitative differences in the estrogen injected, neither latent period nor length of heat shows comparable changes. Animals that were brought into heat 48 hr. after a conditioning dose of 50 I.U. of theelin had a mean latent period of 4.96 hr. and heat length of 6.32 hr.; whereas animals brought into heat 48 hrs. after a conditioning dose of 200 I.U. of theelin had a mean latent period of 3.70 hr. and heat length of 7.56 hr. There is a difference, but it is not proportional to the quantity of hormone; apparently supraliminal doses have little affect on the character of the reaction.

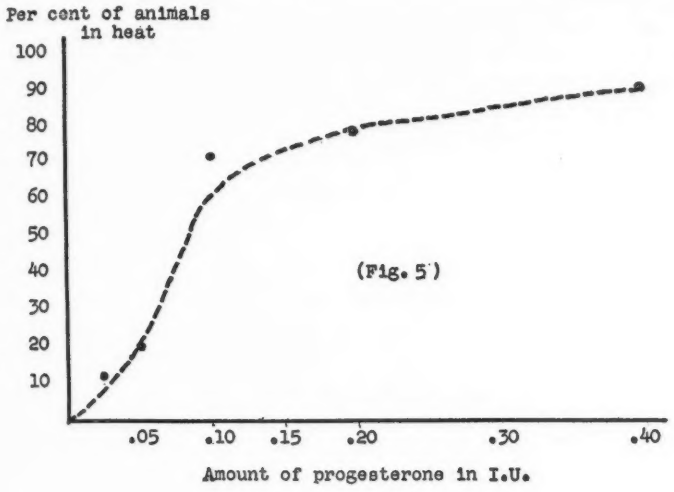
A practical value of this procedure is the use to which the curves may be put in biological testing and assaying. So far, the estrogens used, when followed by progesterone, have induced the estrous response and have given typical frequency distribution curves. Hence, a technic which is simple and subject to rigid quantitative analysis is available for the testing of estrogenic activity. The estrous response test can also be used in assaying the potencies

TABLE 6. EFFECT OF THEELIN DIVIDED INTO 4 DOSES OF 12.5 I.U. ADMINISTERED AT 12-HR. INTERVALS, THE LAST DOSE BEING FOLLOWED 12 HR. LATER BY EITHER, (A) 0.2 I.U. OF PROLUTON OR (B) 0.2 I.U. OF PROLUTON PLUS 50 R.U. INTRAVENOUS THEELIN

Procedure	Number injected	Number in heat	% in heat	Mean latent period	Mean length of heat	Combined latent and length
				Hours	Hours	Hours
(A)	12	11	91.7	2.70	9.30	12.00
(B)	15	14	93.3	3.38	15.95	19.33

of different estrogenic preparations, and the use of figure 6 would enable one to determine the relative strength of different solutions of theelin.

With regard to the corpus luteum hormone, it was demonstrated by



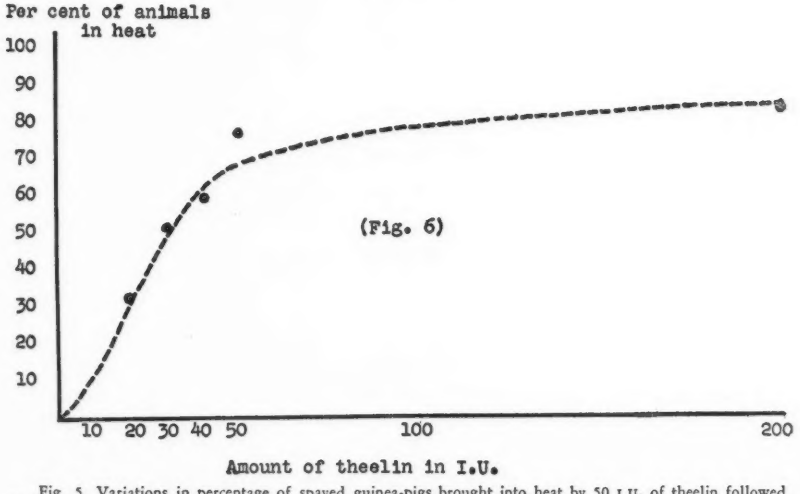


Fig. 5. Variations in percentage of spayed guinea-pigs brought into heat by 50 I.U. of theelin followed 24 hr. later by varying doses of proluton. Fig. 6. Variations in percentage of spayed guinea-pigs brought into heat by 0.2 I.U. proluton after 24 hr. of conditioning by various doses of theelin.

Hertz, Myer and Spielman (5) that in so far as their tests went, progesterone alone is specific for eliciting the copulatory response and completing the estrogen conditioning. In so doing, they varied the amount of progesterone following divided estrogenic dosage. Since their results are quite uni-

form, they propose the estrogen-progesterone reaction as a means of assay for progesterone, using the number of animals reacting as an index to the quantity of hormone present. We have modified their procedure by using single conditioning doses of estrogen followed by the test solution of progesterone; then by referring the percentage of animals reacting to data shown in figure 5, one can determine the quantity of hormone present.

The unusually long heat obtained following intravenous injection of theelin is of interest in connection with the observation that prolonged heat in the guinea-pig is accompanied by delayed ovulation (6). The experimental results suggest that in such cases the availability of large quantities of estrogen about the time of ovulation is an important factor causing this type of heat. The suggestion is offered as one point of departure for an investigation of the entire problem of heat length.

SUMMARY

Experimentally induced heat in the guinea-pig can be put on a rigid quantitative basis with reproducibility of results. Animals may be kept conditioned for 5 or more days with single injections of theelin and for 8 or more days with progynon-B. The latent period and length of heat are inversely related and for a given procedure their sum approximates a constant. The latent period does not increase nor the length of heat decrease in animals brought into heat after long periods of conditioning. An 'all-or-none' mechanism of sexual receptivity is postulated. Supraliminal doses of theelin or of progesterone do not significantly change the length of heat nor the latent period, except when intravenous injections of theelin are given. Curves for the biological testing and assaying of estrogens and of progesterone are given.

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PARTIAL INHIBITION OF SEX ACTIVITY IN THE INTACT FEMALE RAT BY INJECTED ESTRIN[1]

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Estrin, injected into the castrated female, produces the anatomical and behavior changes characteristic of estrus. But if estrin is injected into the intact female in adequate amounts daily for some time it prevents the ovarian changes upon which normal estrus is dependent. The literature on this subject is too extensive to review here. The most comprehensive investigation of the effect on the ovary of doses comparable with those used in the experiment reported below was made by Button and Miller (1) who review the more important papers concerned with small daily doses of estrin. None of the investigators studying the anatomical effects, however, make any report of behavior. It seemed possible that the hormone which produces sex behavior in the castrate might be used to prevent it in the intact animal. The following experiment was made to test this possibility.

EXPERIMENTAL PROCEDURE

Eight rats about 3 months old were used in this experiment. Their sexual excitability was measured each heat period for one month before injections were started. In all but one case it was found to be 'normal'; i.e., it was rated 7 or more on a scale of 12 points which has been described elsewhere (2). The one animal that was never rated more than 2 during these untreated cycles differed during the experimental period only in needing less estrin to prevent the return of cyclic behavior and vaginal smears. Behavior tests were made at 7, 9, 11 P.M. and 1 A.M.

Estrin, in the form of progynon-B,[2] was injected in varying doses for 2 months, at first daily, later every second day. Since it was the purpose of the experiment to find, if possible, a dose that would repress the ovaries and yet be insufficient in itself to directly stimulate mating behavior, the amounts injected were adjusted for each rat from time to time, being at first decreased from an original 5 R.U. daily to ½ R.U. every other day. When this low dose was found to permit the return of cyclic variation in behavior and smears, it was increased because, although Button and Miller (1) have shown that the ovary may be repressed by doses of estrin which permit vaginal cycles, sex behavior in my animals tended to return to normal, with peaks of excitability

[1] This investigation was supported in part by a grant from the Committee for Research in Problems of Sex, National Research Council.

[2] Grateful acknowledgment is made to the Schering Corporation for the progynon-B used in this experiment.

sometimes as high as it had been before estrin administration, whenever vaginal cycles returned. When the dose had been increased to 1 to 3 R.U. every second day and the animals, after 1 and 2 weeks on these doses, were again beginning to show cycles, injections were discontinued.

During the brief recovery period observations were continued. They were mated in their first subsequent heat period to determine whether the entire reproductive functions had returned to normal. Vaginal smears were taken throughout the experiment. Sex behavior was tested at least once every night during the injection and recovery periods until the animals were bred. These tests were made between 9 P.M. and 1 A.M. The method of testing has been described elsewhere (2).

RESULTS

Vaginal smears during the injection period consisted, typically, of cornified cells, except that there was a tendency for normal cycles to return. These were indicated by the return of leucocytes in the smears and refusal to mate, followed by estrous smears and return of sex activity, sometimes to a normal degree of intensity. Smears at these times were not always normal. Sometimes there would be two days of normal diestrous smears, followed by 4 or 5 days or more of estrous smears, and sometimes there would be several days of leucocytes with cornified cells also. The behavior was more definitely cyclic than the smears.

Only once was there a long period of diestrous smears such as Button and Miller (1) report. This was at the beginning of the injections when one animal showed leucocyte smears for 10 days, with 5 R.U. of estrin daily, and for the following 5 days while she was receiving 2 R.U. a day. This difference in vaginal response is no doubt due to a lower threshold in this strain of rats as compared with that of Button and Miller.

Sex behavior during acyclic smears was somewhat irregular, the animals sometimes consistently refusing vigorously mounting males, sometimes accepting, occasionally darting and crouching a little after copulation. Excitability was, however, always low. No part of the heat behavior pattern was shown before copulation and, except during cyclic vaginal smears, they never reacted in any way to the relatively slight stimulus of an immature male (see 2 for the quantitative significance of this behavior). The ratings assigned during acyclic smears were 0 to 3. There was no relation to the size of the dose of estrin in this variability.

The peaks of excitability, which occurred when cycles were returning, were sometimes as high as they had been before any injections. At other times they were definitely lower. They were never higher than they had been in normal heat periods.

Except when cycles were returning, therefore, sex behavior was on a low plateau throughout the injection period, higher than the absolute refusal to mate characteristic of diestrus but much lower than the behavior that normally occurs at estrus. When cycles started to return, sex behavior as well as vaginal smears became cyclic, falling to zero and then rising well above the acyclic plateau, sometimes to the normal estrus height.

Recovery from the effects of the injections was rapid. One animal was already showing cycles at the end of the injection period and she became pregnant 2 days later. Five of the others had normal heat periods within a week; the remaining two, 11 and 12 days, respectively, after the last injection.

Smears were usually of the diestrous type within a day or two, but cornification lasted 5 to 8 days in 3 animals. Diestrous behavior was fairly synchronous with the diestrous smears, although in one case of continued cornification behavior was normally cyclic, and 2 animals continued to accept the male for 1 and 3 days, respectively, after the smears had become diestrous.

Each of the animals produced normal litters, all but one of them being impregnated the first heat period after the injections were stopped. The first two matings of the exceptional animal were followed by apparent pseudopregnancy, as indicated by smears, but the third resulted in a normal litter of seven young.

It is evident from these results that the injected estrin prevented the high sexual excitability that is produced by the animal's own organs functioning normally, although it produced what would commonly be called 'constant estrus,' since there were estrous smears most of the time, and the animals did usually accept copulation. It is also evident that the injections did not produce lasting damage to the reproductive function.

SUMMARY

Eight adult rats were injected with estrin for 2 months, each animal receiving changing doses which varied from 0.25 to 5 R.U. a day. Cyclic changes in sexual excitability were prevented by this treatment. Behavior during the injection period showed a fairly constant low degree of sexual receptivity, rated 0 to 3 on a scale of 12, whereas the untreated heat periods before and after estrin administration were characterized by peak ratings of 7 to 12 in most cases.

It appears, therefore, that estrin administered in small daily doses is, in a sense, a sex depressant for the intact female rat.

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HISTOLOGIC EFFECTS INDUCED IN THE ANTERIOR PITUITARY OF THE RAT BY PROLONGED INJECTION OF ESTRIN WITH PARTICULAR REFERENCE TO THE PRODUCTION OF PITUITARY ADENOMATA[1]

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The reaction of the anterior pituitary to injection of estrin, administered for relatively short periods of time, is fairly well known. Such injections induce an increase in the weight of the gland (1, 2, 3), which is more or less proportional to the amounts injected and the period of administration. Histologically (3, 4), injection of estrin for short periods induces degranulation of the basophiles and a decrease in the level of these cells in the gland. Many eosinophiles also present evidence of granular loss, resulting in a reduction in the number of these cells present. Degranulation of the chromophilic cells is accompanied by hypertrophy of the Golgi apparatus (3, 5). There is a marked increase in the level of the chromophobes; many of these cells become greatly enlarged and the Golgi apparatus is increased in size. Such histologic changes may be considered indicative of an active secretory process, since estrin injections induce the release of a luteinizing hormone from the anterior lobe (6, 7, 8, 9).

More recently the anterior pituitaries of rats and mice, subjected to prolonged injection of estrin, have been studied (10, 11, 12). It was found that the pituitaries were extremely enlarged, and in some instances pituitary adenomata were induced. Only limited histologic studies have been presented. Cramer and Horning (10) reported the presence of chromophobe adenomata associated with massive hemorrhage and congestion. The chromophilic cells were decreased in number and the chromophobes were very abundant. McEwen and associates (11) found 'cavernous adenomata.' Zondek (12) has not yet published a histologic analysis of his material.

In the studies recorded below, we have made a histologic study of the reaction of the anterior hypophysis to injection of estrin[2] for periods of time ranging from 30 days to over 1 year. Cell counts were made on all glands studied. Briefly, we have found that such injections induced hypertrophy of the gland which was associated with adenoma formation in those rats receiving injections for the longest periods. Histologically, there was a generalized

[1] These studies were aided by a grant from the International Cancer Research Foundation.

[2] Dihydroestrin-benzoate, progynon-B, was kindly furnished by the Schering Corporation through the courtesy of Dr. Erwin Schwenk.

degranulation of the chromophilic cells and marked increase in the chromophobes throughout the gland. In rats injected for prolonged periods this generalized reaction was replaced by more localized reactions in different portions of the gland, i.e., localized adenomatous areas were found. Three different types of circumscribed or vaguely circumscribed lesions which we have tentatively termed adenomata have been observed.

EXPERIMENTAL RESULTS

Series 1. Ten female rats, half of which were castrated at the beginning of the experiment, received injections of 200 R.U. of estrin daily for 30 days. At autopsy the pituitaries were markedly increased in weight, the mean being 30.2 mg. (table 1). The ovaries of the 5 non-castrated rats contained large active corpora lutea, indicating that the release of a luteinizing factor by the anterior lobe, stimulated by the injection of estrin, had persisted for this

TABLE 1. SUMMARY OF QUANTITATIVE DATA ON PITUITARIES OF RATS RECEIVING INJECTIONS FOR 30 AND 80 DAYS. FOR COMPARISON, SIMILAR DATA PREVIOUSLY PUBLISHED ARE GIVEN ON NORMAL FEMALE RATS AND RATS INJECTED FOR 10 DAYS

Type of Animal	Rats Used	Av. Weight	Pit. Weight	Percentage of Cells				Mitoses per Section
				Eosinos.	Gran. Basos.	Non-Gran. Basos.	Chrom.	
Normal	143	48.1	10.2	34.2	1.2	2.9	61.7	1.2
Estrin—10 days	41	64.6	20.7	23.3	—	1.0	75.8	36.6
Estrin—30 days	10	61.2	30.2	15.8	—	0.1	84.1	26.1
Estrin—80 days	10	36.0	33.3	14.1	—	0.1	85.8	10.1

period. The vaginae were mucified. Histologically, in the anterior hypophysis it was found that the basophiles were completely degranulated; the level of these cells was extremely reduced. The eosinophiles were markedly reduced in percentage when compared with the glands of normal females (table 1). Many of these cells showed evidence of granular loss, i.e., these were somewhat swollen, the granular content was reduced and the Golgi apparatus, judging by the appearance of its negative image, was hypertrophied. The chromophobes were markedly increased in percentage (table 1). Many of these cells were enlarged. In both the large and small chromophobes the Golgi apparatus was increased in size. In some cells the cytoplasm was a dense blue; in others it was lighter and fragmentary in nature (fig. 3, 5). In many cells small yellowish-orange bodies, considered to be mitochondria, were observed. Mitoses were abundant; the mean number counted per section was 26.6 (table 1). A great majority of these were found in chromophobes, although a few were found in eosinophiles. The anterior lobes of both the castrated and non-castrated rats were equally affected by these injections.

In many areas of these glands the capillaries were widely dilated. However, they contained relatively few red cells (fig. 4). These markedly distended capillaries separated narrow cords of anterior lobe cells, most of which were chromophobes. Generally the degree of hypertrophy of the anterior lobe cells

was less marked in the regions where the capillaries were widely dilated than in the areas where vascularity was less marked (fig. 4, 5).

Another condition was noted in these glands which has not previously been described. In many cells, small clear vacuoles were observed (fig. 4, 6). For the most part this condition was generalized throughout the gland, but in some instances only isolated groups of cells were affected. In some cells the vacuoles were single, while in others they were multiple. This vacuolar change appeared to be degenerative in character and in most instances affected chromophobes. Although special studies have not been made to determine the nature of the contents of the vacuoles, the change has all the characteristics of fatty degeneration.

Series 2. A second group of 10 female rats, half of which were castrated, received subcutaneous injections of 200 R.U. of estrin daily for 80 days. The ovaries of the non-castrated rats were small (table 1), and upon histologic examination were found to contain small regressing corpora lutea and small follicles, most of which were atretric. In both the castrates and the non-castrates the vaginae were either stratified or stratified and cornified. The uteri of 8 rats were lined by pseudostratified epithelium; in 2 instances squamous metaplasia of the uterine epithelium was observed.

The pituitaries were only slightly heavier than those of rats which received estrin for 30 days (table 1). Histologically, the anterior pituitaries of this group were so similar to those which received estrin for 30 days that a separate description is not needed. Quantitative data are summarized in table 1. However, it was found that the frequency of mitoses was decreased and the degree of vacuolization of the anterior lobe cells was somewhat greater. In many glands large focal masses of colloid were present between the anterior lobe cells (fig. 7). Again, the reaction obtained in castrated and non-castrated rats was identical.

Series 3. In this series 2 groups of rats were used. The first was composed of 9 rats which received daily injections of 200 R.U. of estrin for a period of time ranging from 180 to 425 days. Both males and females, half of which were gonadectomized, were used. Eight castrated female rats comprised the second group. They received interrupted injections of estrin, specifically, daily injections of 200 R.U. for 10 days followed by a rest period of 20 days. This procedure was repeated 8 times; the animals were killed 247 days after the experimental regime was initiated. Rats used in these 2 groups are listed individually with pertinent data in table 2, and are considered as a single series.

In this series 2 different types of cellular reaction in the anterior lobe were observed. In 8 rats the reaction was generalized or diffuse, a uniform histologic structure being found throughout the whole gland. Nine rats presented adenomatous pituitaries. In various portions of the gland from one to several nodular foci of cells with structural characteristics differentiating them from the surrounding tissue, were found.

The pituitaries of the rats presenting the generalized reaction were markedly increased in weight, the range being from 43 to 85 mg. (table 2). The

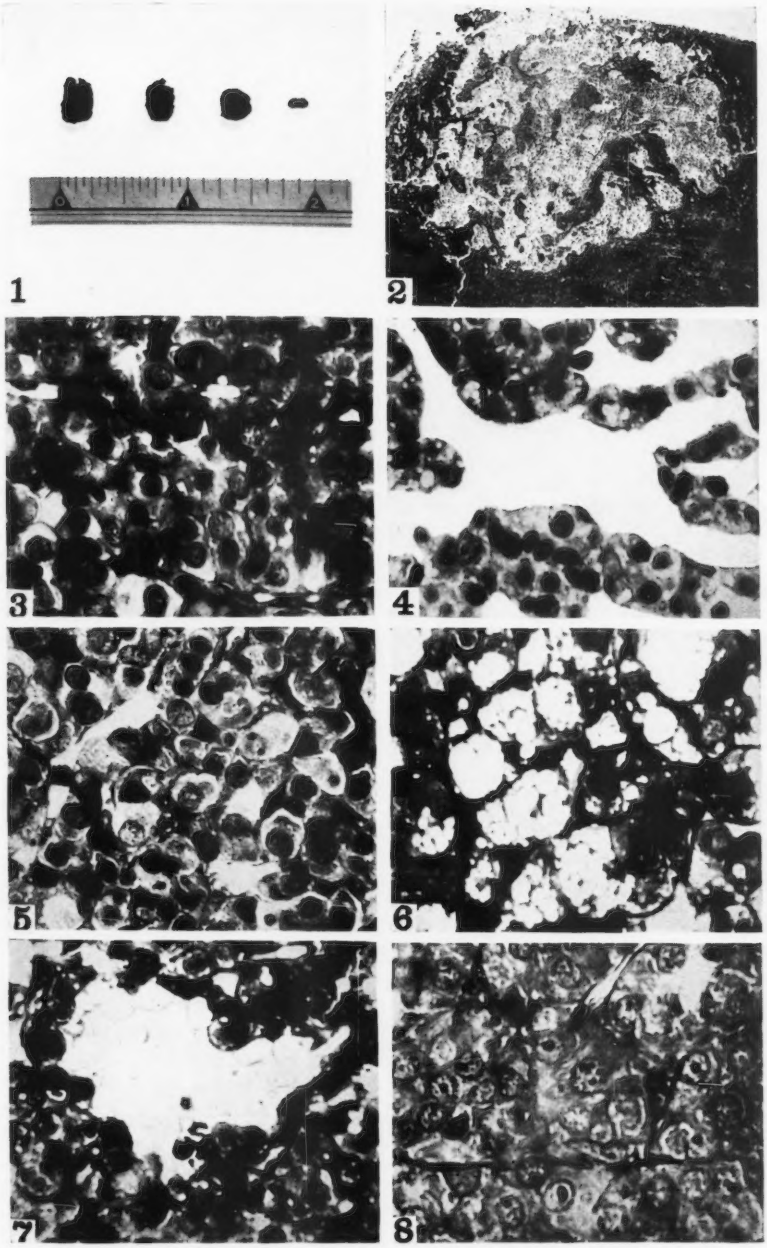
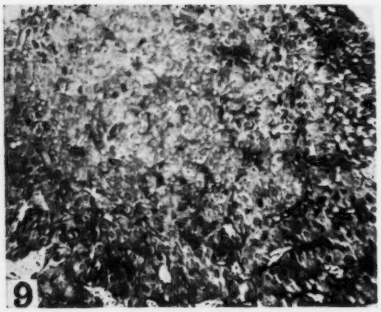
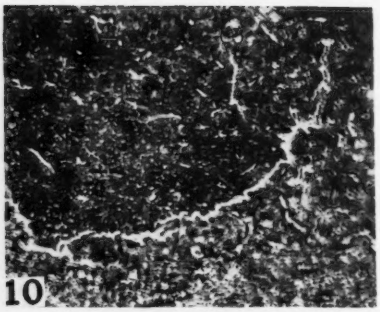
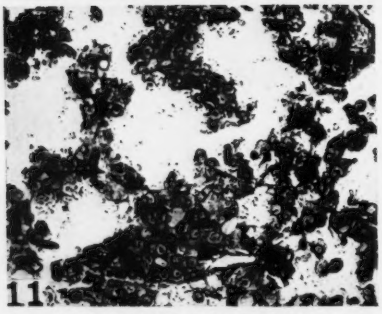
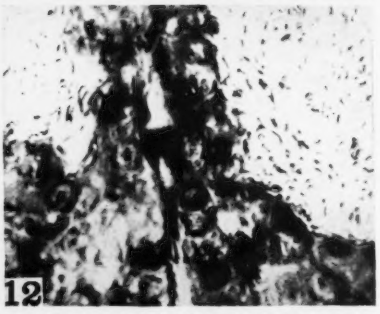


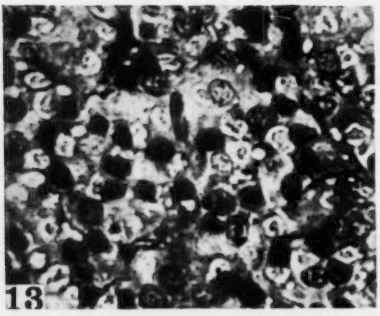
Fig. 1. The pituitaries of rats *3459*, *3460* and *3405* are pictured from left to right. On the extreme right is a normal pituitary which weighted 12 mg. Photographed slightly less than normal size. Fig. 2. A hemorrhagic chromophobe adenoma. x30. Fig. 3. Section through anterior lobe of normal female rat. x390. Fig. 4. Through anterior lobe of injected rat, showing marked capillary dilatation. x390. Fig. 5. Through anterior lobe of rat injected for 10 days. x390. Fig. 6. Showing extreme vacuolization of anterior lobe cells. x390. Fig. 7. Showing mass of intercellular colloid. x390. Fig. 8. Showing generalized reaction in anterior lobe of rats injected for 1 year. x390.

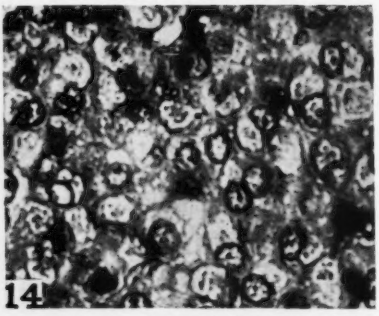


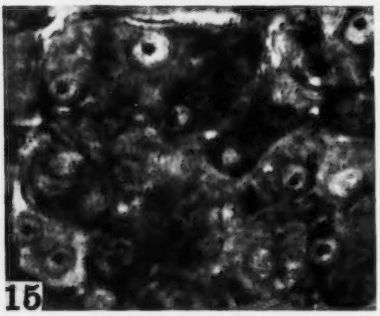












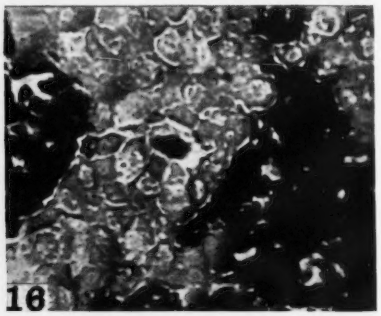


Fig. 9. Section showing nodular focus of markedly hypertrophied eosinophiles. x135. Fig. 10. Showing nodular focus of small chromophobe cells. x135. Fig. 11. Showing cords of anterior lobe cells projecting into wide blood spaces in a hemorrhagic chromophobe adenoma. x240. Fig. 12. Showing a single cord of cells projecting into a blood-filled space in a hemorrhagic chromophobe adenoma. Note normal capillary between the two rows of cells. x390. Fig. 13. Through a nodular focus made up of small chromophobes. x390. Fig. 14. Through chromophobe adenoma in rat 3405. Fig. 15. Through a nodular focus made up of large eosinophiles. x390. Fig. 16. Through a hemorrhagic chromophobe adenoma. The filters were arranged to emphasize the red blood cells. x390.

eosinophiles exhibited marked degranulation and were reduced in relative percentage to levels as low as 4.2 per cent. Many individual cells showed marked hypertrophy and an increase in the size of the Golgi apparatus. The basophiles had undergone complete degranulation; by the staining technique used in this study only rarely were basophiles demonstrable. The chromophobes were markedly increased in number; the highest level found was 95.8 per cent (table 2). These cells varied considerably in appearance. Some were small, had scant sytoplasm and dark-staining nuclei, and appeared relatively quiescent. However, a majority showed varying degrees of hypertrophy; in these cells the Golgi apparatus was increased in size, sometimes to a marked extent (fig. 8). In many of the larger cells the nuclei were large and pale and the nucleoli appeared particularly large and prominent (fig. 8). In many cells small, yellowish-orange bodies were abundant in the region of the Golgi apparatus; these were considered to be mitochondria. In some areas the capillaries were markedly distended, resulting in a wide separation of the cords of the anterior lobe cells in other areas the cells were arranged quite close together (compare fig. 4, 8). In all these glands, varying degrees of the vacuolar condition of the cells were encountered.

Mitoses were found in practically all glands but the numbers counted per section were not excessive (table 2). In practically all glands many masses of intercellular colloid were seen (fig. 7). In most instances the residual cleft was packed with colloid and there was some compression of the intermediate and posterior lobes. Study of this material has convinced us that the weight and histologic reaction obtained were an intensification of that obtained in the glands of rats receiving estrin for periods of 30 or 80 days.

In 9 rats a pituitary reaction which we have considered as adenomatous, was obtained (table 2). Some of the glands were extremely heavy, weighing up to 248 mg. (table 2). They were variable in appearance. Two glands (*3359* and *3360*) were decidedly abnormal in shape, being greatly increased in their antero-posterior diameter (fig. 1). The intermediate and posterior lobes were not grossly recognizable. In 2 glands (*2685* and *3406*) irregular and deeply congested secondary swellings were encountered in the right lateral half of the gland. In 1 of these (*2685*) the intermediate and posterior lobes were not seen. The gland of rat *3405* was uniformly swollen (fig. 1) but intermediate and posterior lobe tissue was not discernible. The glands of all other rats were uniformly enlarged; the intermediate and posterior lobes were grossly visible.

Before presenting a description of the various types of adenomata encountered, it should be pointed out that an extreme reaction to estrin was obtained in the extra-adenomatous areas of these glands. Basophiles were not found in any of them. The degranulation of the eosinophiles was profound, in 1 animal being complete. Associated with the almost negligible levels of chromophilic cells were tremendously high levels of chromophobes (table 2). Numerous large masses of intercellular colloid were found. Vacuolar degeneration of the cells was extreme in some instances. The histologic reaction in the extra-adenomatous portions of these glands represented a considerable

TABLE 2. DETAILED QUANTITATIVE DATA ON THE ANTERIOR PITUITARIES OF RATS RECEIVING ESTRIN FOR PROLONGED PERIODS

Rat Number	Type of Animal	Injection Period	Rat Wt.	Pit. Wt.	Percentage of Cells			Mitoses per Section	Types of Pit. Reaction
					Eos.	Basos.	Chrom.		
K-533	Female Cast.	Interrupted 284 days	210	48	5.6	—	94.4	7	Generalized
K-599	Female Cast.	Interrupted 284 days	260	87	4.2	—	95.8	9	Generalized
K-600	Female Cast.	Interrupted 284 days	250	48	7.1	—	92.9	10	Generalized
3418	Female Cast.	Interrupted 247 days	226	85	11.8	—	88.2	17	Generalized
3419	Female Cast.	Interrupted 247 days	215	43	10.5	—	89.5	6	Generalized
3359	Female Cast.	Interrupted 247 days	221	248	0.9	—	99.1	46	Adenomatous
3360	Female Cast.	Interrupted 247 days	225	156	2.2	—	97.8	28	Adenomatous
3420	Female Cast.	Interrupted 247 days	250	80	6.9	—	93.1	12	Generalized
3405	Female Cast.	Daily 348 days	256	144	5.0	—	95.0	19	Adenomatous
3406	Female Cast.	Daily 238 days	195	69	9.5	—	90.5	2	Adenomatous
3407	Female Normal	Daily 186 days	212	45	8.5	—	91.5	4	Generalized
3408	Female Normal	Daily 232 days	180	34	11.8	—	88.2	6	Adenomatous
3409	Male Cast.	Daily 257 days	267	38	12.6	—	87.4	10	Adenomatous
3410	Male Cast.	Daily 325 days	260	50	11.0	—	89.0	27	Adenomatous
3411	Male Normal	Daily 132 days	210	45	12.2	—	87.8	13	Generalized
3412	Male Normal	Daily 306 days	306	41	9.7	—	90.3	11	Adenomatous
2685	Female Cast.	Daily 425 days	246	139	0	—	100.0	2	Adenomatous

intensification of that found in the glands of this series presenting a generalized reaction.

At least 3 different types of pituitary adenomata were found. In 2 rats (*2685* and *3406*) prominent adenomata associated with marked vascular changes and hemorrhage were noted. These have been designated as hemorrhagic chromophobe adenomata by Cramer and Horning and from our observations we feel that this term is appropriate. In rat *2685* the adenoma

measured approximately 2 x 3 mm., invading the whole lateral half of the gland; in rat *3406* the adenoma was much smaller (fig. 2). They were fairly well circumscribed; at their periphery there was considerable compression of the adjoining cells of the extra-adenomatous tissue. The anterior lobe tissue in the adenomata was thrown into long thin cords, usually comprised of only 2 layers of cells which projected into wide sinusoid-like spaces which were packed with red blood cells mixed with small amounts of fibrin (fig. 2, 11, 12, 16). Of particular interest is the fact that these wide blood-filled spaces did not possess an endothelial lining but were lined by anterior lobe cells (fig. 11, 12) which in some instances were either cuboidal or flattened in shape. The blood-filled spaces were thus actual hemorrhages. The origin of these enormous hemorrhages was not ascertained; in some places it appeared that normally lined capillaries opened into the blood spaces, but this was not a constant finding. On the other hand, normal but narrow capillaries were found between the rows of cells which constituted the narrow cell cords which projected into the lakes of blood (fig. 11, 12). Near the periphery of the adenomata smaller interstitial hemorrhages were often found. Associated with the marked hemorrhages in these adenomata were abundant large hemosiderin-filled clasmatocytes.

These adenomata were comprised exclusively of moderately hypertrophied chromophobe cells. The Golgi apparatus was enlarged but the cells were not appreciably different from chromophobes found in the extra-adenomatous areas of the gland (fig. 12). In these two glands mitoses were rare, both in the adenomata or in the extra-adenomatous tissue.

In 2 other glands (*3405* and *3410*) small localized areas were found which we considered to be the starting point of hemorrhagic chromophobe adenomata. In both there were several rather small connecting spaces lined by anterior lobe cells and packed with red blood cells. Clasmatocytes containing hemosiderin were also observed. In one gland this process was apparently beginning over a fairly wide area, and there was already some slight compression of surrounding anterior lobe tissue. We feel that this compression of the surrounding anterior lobe cells is due chiefly, if not entirely, to the pressure exerted by the interstitial hemorrhage, because in these hemorrhagic, chromophobe adenomata we have found no evidence of any rapid increase in the number of cells.

In rat *3405* a moderately large (1.2 × 1.2 mm.) chromophobe adenoma was found. It was well delineated from the surrounding tissue by a definite compression of several rows of the adjoining anterior lobe cells, each row being more or less separated by a collapsed capillary and its fragile surrounding tissue. This tumor was made up of hypertrophied chromophobes which were markedly uniform in appearance (fig. 14). In all, the Golgi apparatus was extremely hypertrophied, almost entirely filling the extra-nuclear portion of the cell. The region of the Golgi was well filled with coarse, orange-colored bodies considered to be mitochondria. This adenoma was relatively avascular, only occasional capillaries being observed. In 1 area, however, several large blood spaces which were lined by anterior lobe cells were found. It seems

quite possible that this represents an initiation of the transformation of a relatively avascular adenoma into one of the hemorrhagic types. Mitoses were abundant throughout this adenoma; in the extra-adenomatous area of this gland, they were much less frequent.

In 3 rats (*3408, 3409, 3410*) small, vaguely outlined, nodular foci of cells were found which were different in appearance from those of the surrounding tissue. Two types of such nodular foci were observed. The first type was made up of chromophobes much smaller and much more closely packed than the cells of the surrounding tissue (fig. 10). These small chromophobes presented a markedly hypertrophied Golgi apparatus (fig. 13). At the periphery of such areas there was usually considerable intermingling with the surrounding anterior lobe tissue, but nevertheless they stood out as discrete and easily recognizable bodies when viewed under low power of the microscope. These small chromophobe nodular foci were found in all 3 glands and were multiple in each instance.

The second type of nodular foci was made up of enormously hypertrophied granular cells. Single foci of such cells were found in rats *3408* and *3410*. One of these foci measured 0.2 x 0.2 mm. and was made up of eosinophiles which measured up to 25 x 25 γ (fig. 9). They were well packed with yellow granules, the Golgi apparatus was markedly hypertrophied, the nuclei were pale and contained unusually large and prominent nuclei. The second of these foci was larger, measuring 0.3 x 0.4 mm. At the periphery it was fairly diffuse, the cells mixing somewhat with those of the surrounding tissue. The cells making up this nodular focus were extremely large and contained granules which varied from red to orange. The outstanding features of these cells were the pale nuclei and the large nucleoli (fig. 15). The cells were closely packed together, the cell membranes often being indefinite. Although we can make no positive statement, we believe that these cells were markedly hypertrophied eosinophiles which exhibited an abnormal staining reaction, since in this gland we could find intermediate variations between these large cells with red granules and typical eosinophiles.

The glands of rats *3459* and *3460* were the largest obtained in our series, weighing 248 and 156 mg. respectively. They were markedly increased in their antero-posterior dimensions (fig. 1) and grossly did not in any way resemble pituitary glands. In gland *3459* there was a deep cleft extending into the organ from its posterior surface and lying in the antero-posterior direction. Intermediate and posterior lobe tissue was not visible.

Histologically, these glands presented a generalized but somewhat unusual reaction to the injections. They were made up almost entirely of medium-sized chromophobes. Practically all of these contained a markedly hypertrophied Golgi apparatus. The cells were remarkably uniform in appearance and size, although in some cells the nucleus was large and irregular in shape and the nucleolus was remarkably large. Practically all the capillaries were collapsed, the anterior lobe cells all lying in close apposition. Intermediate and posterior lobe tissue was not present; in rat *3460* there was some evidence of remains of the residual cleft. Mitoses were fairly abundant, particularly in gland *3459*. Numerous small masses of intercellular colloid were observed.

DISCUSSION

There is some discussion in the literature as to just what constitutes an adenoma of the anterior lobe. Using human material, Costello (13) has called attention to certain characteristics that influence us in the interpretation of our findings. He points out that a capsule is not necessarily present but that there is often condensation of the surrounding tissue. This we have observed in 3 instances. The term 'hemorrhagic chromophobe adenomata,' used to designate one type of adenoma described by us, seems justified because of the localized circumscribed character of the growths and the fact that they were made up of cells which were almost exclusively of one type, namely chromophobes. Moreover, the cells themselves were atypical in their arrangement, especially with regard to their relationship to local capillaries. Although the reason for their development is not clear, massive interstitial hemorrhages occurred.

Whether or not the small nodular foci of cells described in rats *3408, 3409* and *3410* should be considered true adenomata is open to question. While they were fairly discrete, the cells constituting them intermingled considerably with those of the surrounding tissue. There was no evidence of compression of the adjacent uninvolved tissue. Cells similar to those constituting these foci could be observed elsewhere in the gland, but in the areas described these cells were so concentrated as to give the area a definite nodular and circumscribed appearance. Furthermore, they were practically pure in constitution, being made up almost exclusively of one cell type. Regardless of the fact as to whether or not they should be considered adenomatous, their presence is unusual in the anterior lobes of the rat; we have never observed them before except in the anterior lobe of old, breeding, female rats (14).

The 2 enormously hypertrophied pituitaries of rats *3459* and *3460* were decidedly abnormal in shape. They were increased in weight approximately 15 to 20 times that of the normal and the intermediate and posterior lobes were not demonstrable either grossly or histologically. In spite of the fact that they presented a generalized reaction to estrin, they should probably be considered as a form of diffuse adenomatous hyperplasia because of their extreme size and irregularity in shape.

It seems clear that the initial reaction of the anterior lobe to the injection of estrin is a generalized one. The pituitary gland increases in weight but the hypertrophy of the anterior lobe is uniform. The chromophilic cells undergo degranulation but this process is uniform throughout the whole gland. It is only after prolonged stimulation that gross irregularities in shape and appearance are observed and that adenomatous foci may be found upon histologic examination.

We have recently pointed out (14) that adenomata similar in structure to the hemorrhagic chromophobe adenomata described in this paper may appear spontaneously in the anterior lobes of old female rats. We have also found in these animals the small nodular foci of cells described. This similarity in adenomatous growths which occur in the pituitaries of rats receiving estrin and those which occur spontaneously in old animals seems to be of considerable interest, especially since we have found that the estrous cycles of old

female rats become somewhat irregular, indicating some disturbance of the normal hormonal balance regulating the anterior lobe-gonadal relationship.

SUMMARY

The pituitaries of 37 rats receiving injections of estrin for periods of from 30 to 425 days were studied. In rats treated for the shorter periods such injections induced a marked but uniform hypertrophy of the gland, but in rats injected for prolonged periods adenomata were induced. Histologically, it was found that the initial reaction to injections of estrin was generalized. The chromophilic cells were markedly degranulated and reduced in number, while the chromophobes were markedly increased in size and number. Degranulation of the chromophilic cells was accompanied by hypertrophy of the Golgi apparatus. In 9 rats injected for prolonged periods the generalized histologic reaction was replaced by one characterized by the appearance of adenomatous areas of cells. Three types of adenomata were described. These included pituitary adenomata associated with profound vascular changes, small nodular foci of cells presenting structural characteristics different from the cells of the surrounding extra-adenomatous tissue, and diffuse adenomatous hyperplasia of the entire gland. A vacuolar condition of the anterior lobe cells, considered to be degenerative, was described. Mitotic figures were generally more abundant in rats injected for shorter periods.

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CELLULAR CHANGES IN THE ANTERIOR HYPOPHYSES OF VITAMIN-A DEFICIENT RATS

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That a lack of vitamin A in the animal diet results in sterility is a well-established fact. In this paper, and in others which are to follow, the authors are concerned with the modus operandi of vitamin-A deficiency in producing the pathological and physiological changes coincident with sterility in both sexes. The deficiency may act directly in causing damage to the gonads themselves or indirectly through a disruption of an endocrine relationship in which the gonad becomes non-functional and undergoes atrophic changes because of dietary damage of the endocrine gland or glands from which its stimulating principle emanates.

Mason and Wolf (1) have produced physiological evidence that vitamin A deficiency has a direct damaging effect on the reproductive organs. They compared the gonad-stimulating capacities of the hypophyses of castrate and non-castrate vitamin-A deficient animals with litter-mate castrate and non-castrate animals on an adequate diet. The ovaries of the test animals which received hypophyseal transplants from vitamin-A deficient non-castrates were 43 per cent greater in weight than those which received transplants from normal stock controls. The ovaries of the test animals receiving transplants from vitamin-A deficient castrates were 39 per cent greater in weight than those receiving transplants from vitamin-A deficient non-castrates and 100 per cent greater than those receiving transplants from normal controls. It would appear that the increase in gonad-stimulating properties of the pituitaries represents an attempt on the part of the pituitary to compensate for the direct damage of the dietary regime on the gonad.

Such a marked change in the physiological activity of the pituitary glands should be accompanied by a structural or cytological change in the pituitary of sufficient magnitude to be quantitatively studied. The present paper is concerned with such a study.

The classical work of Wolbach and Howe (2), in which they made an exhaustive study of the pathology of vitamin A deficiency, reported a shrinkage of the gland cells of the pituitary but "no change in the architecture of the gland." Thatcher and Sure (3) reported no changes in the hypophysis in vitamin A deficiency. It is possible that these investigators were looking for epithelial metaplasia in the pituitary which apparently does not occur in vitamin A deficiency.

Mitzkewitsch (4) reported hypertrophy of the pituitary without histological change in vitamin-A deficient rats. It should be noted, however, that this hyper-

trophy was relative to body weight. The pituitaries of the vitamin-A deficient animals were actually much smaller than those from the normal controls. Korenchevsky (5) reported an increase in the number and size of the 'light cells' of the hypophyses of pigeons in vitamin A deficiency. It is probable that both

TABLE 1. DETAILED DATA ON MALE AND FEMALE HYPOPHYSES (ANTERIOR LOBE)

Group	Percentage of different cell types			Group	Percentage of different cell types		
	Chromophobe	Alpha	Beta		Chromophobe	Alpha	Beta
Normal Females				Normal Males			
E813A	52.6	42.5	4.9	N1	46.5	41.6	11.6
E814A	60.4	34.8	4.9	N3	46.4	44.2	9.3
E837	51.1	42.3	6.6	N4	46.4	42.0	11.6
E838	55.4	38.7	5.9	N5	46.8	43.5	9.8
E311	53.2	40.5	6.2	N6	46.1	44.2	9.8
E789	54.7	39.2	6.1	N7	47.0	43.5	9.5
E812	57.9	37.0	5.0	N8	47.8	43.3	8.9
E825	51.4	39.9	8.7	N9	43.8	44.2	10.0
E826	54.4	37.2	8.4	N10	46.1	45.1	8.8
E814R	53.1	39.4	7.5	N11	47.6	43.7	8.7
Mean	54.4	39.1	6.4		46.6	43.5	9.8
Vitamin-A Deficient Females				Vitamin-A Deficient Males			
E1227	47.4	39.0	13.6	E1228	38.9	36.9	24.2
E1230	44.8	44.0	11.2	E1229	42.5	36.7	20.8
E1231	43.7	42.3	13.9	E1235	40.9	41.6	17.6
E1232	42.4	45.3	12.2	E1257	41.5	37.6	20.9
E1234	43.8	42.3	13.9	E1259	42.9	35.7	21.4
E1236	40.4	43.5	16.0	E1261	47.4	35.2	17.4
E1262	48.8	38.9	12.2	E1268	38.6	37.5	27.9
E1263	51.6	39.2	9.2	E1284	41.9	37.9	20.2
E1267	50.1	39.7	10.2	E1291	41.1	35.4	23.4
E1271	47.7	38.5	13.7	E1313	40.8	38.5	20.6
Mean	46.1	41.3	12.6	Mean	41.6	37.3	21.1
Castrate Females				Castrate Males			
C-3	40.5	38.1	21.5	C-9	38.4	39.8	21.7
C-4	41.2	40.7	18.1	C-10	34.6	43.8	21.7
C-5	41.7	40.7	17.6	C-11	35.2	39.1	25.7
C-6	44.3	34.7	21.0	C-12	31.7	43.5	24.8
C-7	43.3	37.8	18.9				
Mean	42.2	38.4	19.3	Mean	34.9	41.5	23.5

Total of cells counted 39,269

chromophobe and basophilic cells are included in the 'light cell' classification. A recent report by Frank (6) states that no macroscopic or microscopic changes occur in the hypophyses of rats fed on a diet deficient in vitamin A. In reviewing the work in this field, the scarcity of data and the inadequacy of the data for the interpretation of the physiological changes becomes evident.

Cellular changes in the hypophyses of castrate rats have been studied by several workers, notably Addison (7), Nukariya (8), and Ellison and Wolfe (9). These workers are in agreement that there is a progressive increase in the basophilic elements following castration. The results of Ellison and Wolfe for animals cas-

trated 30 days are as follows: eosinophils, 42.9 per cent; basophils, 14.2 per cent; castration cells, 6.9 per cent, and chromophobe, 41.2 percent. At this time (30 days after castration) the basophilic cells had reached their maximum percentage.

Wolfe and Cleveland (10) have shown that there are cyclic changes in the cellular elements of the hypophyses of normal females. These changes are qualitative rather than quantitive. Differences in the actual percentage of alpha, beta, and chromophobe cells at the various stages of the cycle are insignificant.

MATERIALS AND METHODS

Twenty-four-day-old weanlings of a closely inbred strain of Mus norvegicus albinus were depleted of vitamin A according to the method of the U.S.P.XI. After depletion, as indicated by cessation of growth, the diet was supplemented with standard-reference cod-liver oil varying in amount from 1 to 1.4 U.S.P. vitamin A units per day, for the duration of the experiment. The period between weaning and depletion was usually about 5 to 6

TABLE 2. SUMMARIZED DATA. PERCENTAGE OF DIFFERENT CELL TYPES IN THE ANTERIOR LOBE OF THE HYPOPHYSIS

Group	Cell type (average per cent)		
	Chromophobe	Alpha	Beta
Normal females	54.4	39.1	6.4
Vitamin-A deficient females	46.1	41.3	12.6
Castrate females	42.2	38.4	19.4
Normal males	46.6	43.5	9.8
Vitamin-A deficient males	41.6	37.3	21.1
Castrate males	34.9	41.5	23.5

weeks. The animals were maintained on the vitamin-A supplemented diet for approximately 7 weeks. These rats, 108 to 112 days old at the termination of the experiment, showed symptoms of extreme vitamin A deficiency by loss of weight, xerophthalmia, muscular incoördination, and respiratory disorders.

The animals used as normal controls were maintained on a complete diet and sacrificed at 95 to 113 days of age. No attempt was made to sacrifice all the normal females at the same stage of the estrous cycle, since Wolfe and Cleveland (10) have shown that quantitative cyclic variations in the cellular elements of the hypophysis are insignificant. The castrates were operated upon at 50 to 56 days of age, just 30 days prior to termination of the experiment.

All animals were sacrificed by exsanguination after preliminary ether anesthetization. The pituitaries of all animals were treated by a technic as nearly identical as possible. After fixation in Zenker-formol solution, the glands were embedded in paraffin and cut at 5 μ, the sections being in the horizontal plane of the gland as it lies in the body. The staining technic was that of Masson, as described by Foot (11), in which Ponceau de Xylidine, acid fuchsin, and anilin blue are used as cytoplasmic stains.

Upon examining the stained sections, it was apparent that certain regions of the anterior lobe contained concentrations of cells of one particular type.

These areas were found to be relatively constant in position in the different glands. We therefore decided to count all the cells on a line through the greater diameter of the section at a tangent to the innermost projection of the posterior lobe. Although this method does not give a true percentile count of all the cells of the anterior hypophysis, it does permit the rapid counting of all the cells on one axis of the gland. By counting the cells along similar axes in the other glands comparisons can be made between the percentages of the various cell types present.

In practice, the technic involves the use of cross-hairs in the microscope ocular and a mechanical stage. The one hair is super-imposed on the gland so as to lie upon the imaginary axis described above. As the slide is moved by means of the mechanical stage in a direction parallel to that of this hair, a procession of cells crosses a second hair, which is at right angles to the first. As the cells pass the intersection of the cross-hairs, they are counted differentially, thus leaving less to the judgement of the observer than is the case when whole fields are counted. Three different sections from the middle of each gland were counted. The counts were made at a magnification of ×970 using an oil immersion objective.

The gonads of both sexes and the uteri and vaginas of the females were sectioned, stained with hematoxylin and eosin, and examined.

OBSERVATIONS AND DISCUSSION

The cells of the anterior lobe of the hypophysis were classified, for the purposes of this experiment, into 3 large groups. We designated as alpha cells those which stained with acid fuchsin, revealing either a granular or an amorphous cytoplasm. These correspond to the 'eosinophiles' or 'acidophiles' in the literature. The beta cells were, in general, larger than the alpha cells, and their cytoplasmic granules stained with aniline blue. These are the 'basophiles' of the literature. The chromophobes are small cells stained poorly by Ponceau de Xylidine.

The detailed results of the counts are presented in table 1 and summarized in table 2.

It should be noted that the large percentile increase in the number of beta cells in the vitamin-A deficient animals does not reflect an equivalent increase in their absolute number since there is some atrophy of the gland. There was, however, an increase in absolute number of beta cells showing a macula. The increase in number of true castration cells was insignificant.

There was a significant increase in beta cells in the vitamin-A deficient members of each sex. This increase approached the condition found in true castrates. Reference to table 2 however, shows a relatively greater increase in beta cells in the vitamin-A deficient male over the normal male than in the vitamin-A deficient female over the normal female. The greater increase may be accounted for on the basis of a more nearly complete destruction of the germinal epithelium of the male gonad. This study presents further evidence that a vitamin-A deficient diet causes direct damage to the gonad, the increase of beta cells representing a compensatory change in the hypophysis.

SUMMARY

A significant increase was found in the 'basophilic' cells in the hypophyses of vitamin-A deficient rats of both sexes. The increase in basophil count was greatest in the males. The results indicate a partial castration effect on the hypophysis in vitamin-A deficiency.

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POST-ADRENALECTOMY DIURESIS

EFFECTS OF CORTICAL EXTRACTS, SALTS AND ESTRONE

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In the untreated adrenalectomized dog there is an initial period of diuresis, associated with changes in electrolyte excretion, sometimes entailing a negative water balance which subsides as adrenal insufficiency becomes pronounced (1, 2, 3). This loss of water by the kidney is not adequate to explain the severe contraction of extracellular fluid characterizing adrenal insufficiency, and the development of adrenal insufficient symptoms is not entirely or necessarily dependent on it (3, 4, 5).

On the other hand, Winter and Hartman (6) found in the cat that there was no diuresis following adrenalectomy, but rather a reduced urine volume, although the water intake dropped relatively more than the urine. They observed the same effects in normal cats by reducing the amount of food to equal that consumed after adrenalectomy.

The results of similar studies on adrenalectomized rats vary widely. Beaird and Swann (7), for instance, found that on a life-prolonging high salt diet, adrenalectomy increased the urine volume by 5 times, resulting in a diuresis more marked than that which follows post-lobectomy, whereas in untreated animals there was an oliguria. The water intake was not stated.

In studies primarily concerned with electrolyte balance, Rubin and Krick (10) found a slight initial transitory diuresis, associated with electrolyte loss, and followed by oliguria. They stress the fact that inanition accounts for part of the symptoms. Sandberg, Perla, et al (8, 9), using a life-sustaining high vitamin diet, found generally that adrenalectomy did not change the water intake but, in association with electrolyte losses, more than doubled the urine output.

Silvette and Britton (11) emphasize the inability of the fasting adrenalectomized rat, showing adrenal insufficiency symptoms, to excrete water even when fluid is administered. The same phenomenon has been observed after heavy fluid overdosage in the mouse (25), dog (12), and rat (13), in association with a marked susceptibility to water intoxication.

It would seem then that in some species and under certain conditions adrenalectomy invokes a high diuresis, whereas in other forms and circumstances the opposite effect ensues. It therefore becomes of interest to find, if possible, the factors which determine whether diuresis or anti-diuresis follows adrenalectomy. In connection with other studies we have had occasion to make observations pertinent to this question in adrenalectomized rats under various conditions.

METHODS AND RESULTS

Young male rats weighing from 150 to 210 gm. were used. They were kept in individual, all-metal metabolism cages, over a funnel 9 inches in diameter and on a 5/16 inch wire mesh. Food and water intake, urine output and weight changes were measured daily. Feces were collected in a second wire

TABLE I. MEAN FIGURES ON FOOD INTAKE, WATER EXCHANGE, AND WEIGHT CHANGE IN NORMAL AND ADRENALECTOMIZED RATS UNDER VARIOUS CONDITIONS

Series	Type of experiment	No.	Food gm. day ± S.E.	Water cc. day ± S.E.	Urine cc. day ± S.E.	Ratio H_2O/ Urine	Weight change gm. day ± S.E.
I	Controls	58	13.5±0.30	21.1±0.51	7.00±0.34	3.01	+2.30±0.28
II	Adrenalectomized	40	9.1±0.48	21.3±0.89	11.30±0.57	1.88	−1.52±0.28
IIa	Adx.[1] (Acute ad. insufficiency)	11	6.7±0.62	17.7±1.71	9.70±1.02	1.82	−3.00±0.39
IIb	Adx. (Chronic ad. insufficiency)	26	10.0±0.40	24.3±0.75	12.04±0.61	2.01	−1.19±0.16
III	Reduced Food	9	5.7	12.7±0.95	5.71±0.72	2.22	−4.74±0.56
IV	Adx.—0.4 cc. Cort. Ext./day[2]	6	11.0	20.5	9.50	2.16	−0.10
V	Adx.—0.5 cc. Cort. Ext./day[2]	7	15.80	26.6	10.80	2.46	+2.79
VI	Adx.—0.4 gm. Char. Adsorbate/day[3]	11	10.9±0.73	24.7±1.95	11.80±1.20	2.09	−0.54±0.65
VII	Adx.—.78 gm. Char. Adsorbate/day[3]	18	14.8±0.37	28.3±1.25	11.50±1.04	2.46	+2.13±0.42
VIII	Normal—drinking 1% NaCl	8	15.2±0.98	50.3±5.66	31.00±5.59	1.62	+2.57±0.55
IX	Adx.—drinking 1% NaCl	9	11.6±0.50	47.4±3.66	27.20±3.14	1.74	+0.81±0.20
X	Normal—drinking 0.1% KCl	7	14.2	25.5	9.90	2.57	+1.79
XI	Adx.—drinking 0.1% KCl	12	8.2±0.92	22.7±2.14	14.10±1.28	1.61	−3.54±0.67
XII	Normal—Low Salt Diet	12	11.3±0.59	18.2±0.89	10.10±0.95	1.8	+1.20±0.31
XIII	Adx.—Low Salt Diet	10	4.7±0.73	13.0±0.96	8.00±0.83	1.63	−3.01±0.49
XIV	Adx.—100 I.U. Estrone/day[4]	10	7.3±0.53	20.4±1.45	12.00±1.15	1.7	−4.2 ±0.64

[1] Adrenalectomized.
[2] Adrenal cortical extract of the Upjohn Company.
[3] Charcoal adsorbate extract of the adrenal cortex prepared by the Schieffelin Company.
[4] Does not include animals dying in less than 4 days.

of smaller mesh placed midway down the funnel. Control records were kept on all animals 7 days or longer, for comparison with later experimental results. There was inevitably a considerable loss of urine by evaporation on the collecting funnel, but the conditions under which figures were obtained were constant for all groups.

Controls. The mean daily food and water intake, urine volume and weight increment for 58 normal rats on our diet[1] is given in table 1, Series *I*. A diet of Purina dog-chow gave almost identical results.

Adrenalectomized, untreated (*Series II*). Observations were made on 40 untreated adrenalectomized rats. They showed on the average a reduced food intake, no change in water intake, and an elevation in urine output. As

[1] This diet contains: GLF Calf Meal, 89%; ground meat scraps, 8.9%; brewer's yeast, 0.39% cod liver oil, 1.66%. Lettuce usually given twice weekly was omitted here.

moribundity developed and death approached, appetite and thirst were generally lost, causing a terminal oliguric period usually of 1 to 2 days' duration. This period is omitted from the average figures quoted here and in subsequent experiments.

The important facts are more clearly shown, however, if these animals are divided into groups, classified according to the severity of their adrenal insufficiency. The rats of Series *IIa* were those which developed an acute adrenal insufficiency, as judged by the fact that they survived not more than 2 weeks. In this group the food intake was low. The water intake dropped slightly below normal levels but was high in relation to the food intake, and there was only a slight mean urine augmentation. In 4 of the rats of this series the urine output remained at normal levels, although the water intake was somewhat reduced. In the others there was a mild diuresis, sometimes more marked during the first 2 to 5 days following adrenalectomy, and persisting until terminal stages. Results found in this group resemble in part those found by Beaird and Swann and Rubin and Krick in rats, and by Winter and Hartman in cats.

Series *IIb* is composed of those animals with a less acute but distinct adrenal insufficiency which lived 3 weeks or longer, some living indefinitely. Continuous records were not kept for longer than 3 weeks, but in most of the animals intermittent observations were made during subsequent periods. The exact mean life-span of these animals is not known, because some were killed later for other purposes. There were no differences in the results between those animals living for a long period after adrenalectomy and those that lived indefinitely, if any of the latter group which failed to show signs of adrenal insufficiency (decreased growth rate, decreased appetite) were eliminated. In Series *IIb* the food dropped less than in Series *IIa,* the water intake increased significantly above normal (an effect opposite that in Series *IIa*) and the diuresis, which apparently may be of indefinite duration, was marked. As far as the urine volume is concerned these results were somewhat similar to those of Sandberg et al (8).

Despite the considerable differences between the absolute figures of Series *II, IIa,* and *IIb,* the ratio of water intake to urine output remained fairly constant throughout, at near 2:1, as compared to 3:1 for intact animals. In no group and in no individual (except at times during the terminal, moribund stages) was there an actual negative water balance, such as is characteristic of the adrenalectomized dog.

Inanition experiments. Normal animals (Series *III*) were put on a reduced diet similar to that eaten by rats developing acute adrenal insufficiency. Rubin and Krick have clearly associated part of the symptoms of adrenal insufficiency in the rat with inanition. The water intake and urine output were lower in normal rats on this diet than in adrenalectomized animals, indicating that, unlike the cat, the effects of adrenalectomy on water balance in the rat are not wholly due to low food consumption.[2] The water intake in the acute

[2] One animal was omitted from the mean calculations because of a completely atypical response. On two different occasions when the food intake was reduced an increased water intake and pronounced diuresis resulted.

cases of adrenal insufficiency is greater than would be expected in normal animals on the same food intake. This indicates, in agreement with Rubin and Krick, that the post-adrenalectomy diuresis in the rat results in some thirst. In the dog such a thirst is not present (14).

Effects of therapeutic and toxic agents. A), Cortical extracts. Thirteen adrenalectomized rats were treated with Upjohn's cortical extract in doses varying from 0.3 to 0.7 cc. per day (Series *IV* and *V*); and 29 animals were treated with charcoal adsorbate extract made by Schieffelin and Co. after the Grollman method, in doses of from 0.3 to 1.0 gm. per day (Series *VI* and *VII*). Life was maintained with no or only slight weight losses in all cases. A 'complete replacement' dose was defined as that which maintained appetite and weight increment within normal ranges. The 'complete replacement' dose varied from 0.4 to 0.7 cc. per day for the Upjohn extract (average 0.5 cc.), and from 0.35 to 1.0 gm. per day for the Schieffelin product (average 0.78 gm.), (Series *V* and *VII*). Effects of doses in which replacement was not complete are shown in Series *IV* and *VI*.[3] Of interest here are those animals showing a 'complete replacement' because, despite every appearance of health an vigor, only in rare individuals could the water exchange with either extract be returned to normal. There was a chronic high water intake and persistent diuresis. In the few cases in which it was tried, this diuresis was not relieved by dosages of more than a gram of charcoal absorbate extracts per day.

B), NaCl (1 per cent) in the drinking water is known to prevent largely the appearance of adrenal insufficiency in the rat. In normal rats this treatment produced in most cases a marked, but exceedingly variable, increase in fluid intake with a consequent diuresis (Series *VIII*). The only significant differences following adrenalectomy were a slight loss of appetite and a decreased growth rate (Series *IX*). On the contrary, others (7) have found that smaller amounts of Na salts added to the drinking water produce a much greater diuresis after adrenalectomy than before.

C), 0.1 per cent KCl in the drinking water, unlike NaCl, exerted a markedly different effect in normal and adrenalectomized animals. KCl is known to be toxic to animals without adrenals, and much importance has been attached to the rôle of the high serum K in adrenal insufficiency (17, 32, 18). In normal rats a slight increase in fluid intake and urine output followed K administration (Series X). In adrenalectomized rats it reduced the life-span to an average of 13.0 days, compared to an average survival of

[3] The incomplete replacement doses noted here (Series *IV* and *VI*) are not to be construed as the minimal life-maintaining figures, which in mean terms are less. Cartland and Kuizenga (15) have presented comprehensive quantitative studies on the Upjohn extract, and incomplete observations indicate that the dosages of this material we need for life-maintenance are even less than those reported by them. Gaunt and Potts (unpublished) have found that rats, adrenalectomized at 30 days of age, and fed only the low-salt diet of Wieser and Norris (19), can be maintained for periods of at least 3 weeks, showing some weight increment, on from 0.2 to 0.3 gm. of Schieffelin charcoal adsorbate extract per day. The minimal life-maintaining dose in adult rats on either a low-salt or normal diet is much more variable, because at present many adrenalectomized adult rats in our laboratory live for extended periods without treatment. We find the 'complete replacement' dose for adults on a normal diet to be a more constant and reliable figure, but one twice or more greater than that needed for life maintenance. The 'complete replacement' doses reported here are nearly minimal.

In all such work it should be emphasized that different environmental conditions, strains, technics, and diets result in widely different quantitative demands for replacement therapy in adrenalectomized rats, just as those differences have probably accounted for the different survival figures reported by many investigators (16, 30).

The authors are indebted to Dr. G. F. Cartland of the Upjohn Co., and to Drs. R. C. Tallman and E. W. Blanchard of the Schieffelin Company for cortical extracts used here.

more than 3 weeks in untreated, adult, adrenalectomized rats. It evoked a variable fluid intake, the mean value of which was not above normal (yet high in view of the low food intake), but produced a severe diuresis, which generally persisted with little or no abatement until death (Series *XI*). If the error in urine collection is taken into consideration, some of the KCl-treated animals developed, especially during terminal stages, an actual negative water balance, which rarely appeared in untreated adrenalectomized rats.

Selye (20) has emphasized that rats adapted to noxious stimuli before adrenalectomy generally withstand such stimuli much better following adrenalectomy. Seven of the 12 rats given KCl received this treatment for 10 days before adrenalectomy, but no differences were noticed in their post-adrenalectomy response.

D), A low salt diet, as used by Wieser and Norris (19) produced differences in water exchange in normal rats resembling those of acute adrenal insufficiency (Series *XII*). In adrenalectomized rats, which had been on this diet from 5 to 7 days before operation (Series *XIII*), it reduced the life-span to an average of 8.5 days. It produced a low ratio of water intake to urine output, with some instances of a negative water balance. Throughout most of the period this low ratio was due more to a decreased water intake than to an actual diuresis. Eight of the 10 rats showed, however, a sharply increased diuresis for the first 3 days after adrenalectomy. This phenomenon, sometimes seen in all groups, but not with the regularity with which it is reported for the dog, was more consistent here than in any other series. The absolute water and urine figures were lower than those found following any other treatment.

E), Estrone and other sex hormones, like the adrenal cortical hormone, were found by Thorn and Harrop (21) to cause a transient retention of electrolytes and fluid by the kidney. The implication is thereby clear; first, that the estrogens might be beneficial to adrenalectomized animals and to Addisonian cases and, secondly, that the beneficial effects of pituitary therapy (22) might be explained in part at least on the basis of increased estrogen production. Schacher, Browne and Selye (23) and Cavanaugh and Gaunt (24) have found, however, that the estrogens are distinctly toxic to adrenalectomized animals, and that observation has been repeated here. Theelin-in-oil[4] 100 I.U. daily, produced no appreciable changes in the water exchange of 2 normal male rats. (The water-retaining effect reported by Thorn and Harrop in dogs could not perhaps have been detected, if present, in our experiments.) The same daily dose of theelin, given to 12 adrenalectomized rats, however, reduced the life-span to an average of 8.5 days, compared to more than 3 weeks for untreated controls, and produced a diuresis comparable to that found in chronic adrenal insufficiency, and greater than would have been expected in untreated animals showing such a short life-span (Series *XIV*).

The toxic action of estrone may well be due to its inhibition of anterior lobe function, since the administration of pituitary substances extends the

[4] We are indebted to Dr. Oliver Kamm, of Parke,Davis & Co. for the theelin used here.

lives of adrenalectomized animals (22).[5] Kroc (31), however, is of the opinion that while estrogens in the doses used here may be toxic to adrenalectomized rats, smaller doses are beneficial.

DISCUSSION

The data reported here and by others lend themselves to certain descriptive generalizations, without any necessary commitments as to causal factors. Severe adrenal insufficiency may result in either an unchanged, a reduced, or only slightly augmented water excretion. Even when that adrenal insufficiency is suddenly precipitated by a high water administration, at least in the absence of salt, a greatly reduced elimination is seen (11, 12, 13, 25). The loss of extra-cellular fluid in those cases can be explained on the basis of internal fluid shifts (26, 4, 27), as, indeed, must be the case in instances where the kidneys were first removed (28, 29), or following intraperitoneal glucose injections (13). Untreated adrenalectomized rats, in the hands of those workers who find a rapid mortality (7, 10; our animals of Series *IIa*), may similarly show only a slight diuresis or none at all. The condition of chronic adrenal insufficiency which may, in the rat at least, be of long or indefinite duration, is characterized by a persistent diuresis. The fact that the response of rats to adrenalectomy differs widely in different laboratories, where in some cases the typical result is a quickly developing, acute adrenal insufficiency, and in others a long-drawn-out chronic adrenal insufficiency, accounts for the absence or presence respectively of diuresis. Similarly, various sustaining therapies, including high dosages of cortical hormone itself, may result in a persistent diuresis. Diuresis, therefore, characterizes the milder forms and stages of adrenal insufficiency under ordinary conditions. If, however, adrenal insufficiency is induced by influences more or less diuretic in nature, i.e., KCl, estrone and a low salt diet, in dosages which permit survival for several days, much water can be eliminated despite the development of acute symptoms. Whether or not an actual diuresis develops, a low ratio of water intake to urine output invariably follows adrenalectomy.

SUMMARY

The food intake, water intake, urine output, and weight changes were measured in adult, male, adrenalectomized rats under a variety of conditions. Rats which developed acute symptoms of adrenal insufficiency within 2 weeks or less following operation showed only slight if any diuresis. The water intake was reduced. The altered water exchange in these cases was not entirely due to inanition. Rats with chronic adrenal insufficiency developed a marked diuresis which apparently could last until terminal symptoms supervened. Cortical extracts in the relatively large doses necessary for maintenance of normal appetite and growth did not completely remedy the altered water exchange. One per cent NaCl in drinking water maintained life and a water ex-

[5] Different results would probably have been obtained if progesterone rather than estrone had been used. Since the above experiments were made, we (Gaunt and Hays, unpublished results) have found that in the ferret crystalline progesterone maintains life and excellent health following adrenalectomy, whereas the estrogens are toxic.

change not significantly different from that induced by the same treatment in normal animals. One-tenth per cent KCl in the drinking water was toxic to adrenalectomized rats and resulted in a diuresis much more severe than that seen in intact animals given the same treatment. A low salt diet in adrenalectomized rats reduced the life-span and, although the urine volume was less than in normal animals on the diet, it was very high in relation to the water intake. Theelin in doses of 100 I.U. per day was toxic to adrenalectomized animals and produced a conspicuous diuresis. A low ratio of water intake to urine output accompanied all post-adrenalectomy conditions.

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RESPONSE TO COLD FOLLOWING DOUBLE ADRENALECTOMY

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The rôle that the adrenal glands play in the maintenance of normal heat production and regulation of body temperature has been indicated in a previous report by Horvath, et al (1), who showed that following destruction of a single adrenal, cold did not stimulate the metabolism as greatly as it had in normal animals. The material presented in this paper presents evidence regarding the effect of removal of both adrenals on the ability of the organism to adjust itself to the effects of one of our most common stresses—that of cold. The methods employed have been previously described (1). Double adrenalectomy was performed under ether anesthesia. Autopsies were made on all animals immediately after death.

RESULTS AND DISCUSSIONS

The total respiratory quotients are not included in the tables. However, the average values for normal animals were: basal, 0.70; in the cold, 0.72; following exposure to cold, 0.72. The average values for completely adrenalectomized rats were: basal, 0.67; in the cold, 0.68; following exposure to cold, 0.66. The significance of these changes in the respiratory quotient cannot at present be satisfactorily explained.

Response to cold. When normal animals were exposed to a lower environmental temperature for a period ranging between 1 and 2 hours, the metabolism determined at 29°C., immediately after removal from the cold was without exception increased (table 1). Horvath et al (1) had previously reported that the stimulating effect of cold was practically identical, irrespective of length of exposure up to 72 hours. The present series of tests with an average increase of 170 calories or 20.4 per cent confirms this previous report.

Double adrenalectomy produced a significant decrease in the basal heat production (average of 10 per cent). This, however, is a considerably smaller change than that reported by other investigators (7). In only 3 animals (7, *10*, *8a*) of the 14 was the amount of decrease within the 25 per cent fall reported by this group. The heat production after adrenalectomy was decreased in all animals but two (*2*, *8*) where the metabolism could be considered unchanged. The change in heat production seemed to be little influenced by the nearness of death, although in general it was less the closer the test was made to the day of death.

After determinations of basal rate these doubly adrenalectomized animals were exposed to an environmental temperature of 4°C. for periods of the same duration as in control tests, and their heat production then measured at 29°C.

Of the 12 animals only 3 (*8*, *10*, *12*) showed any stimulation. Two of these (*10*, *12*) on autopsy had slight amounts of cortical tissue. These 2 animals studied on another occasion showed the same lack of stimulation observed in the other 9 rats. The decreased calory production varied from 0.2 to 45.7 per cent. The average decrease in heat production in doubly adrenallectomized rats following exposure to cold was 10.6 per cent.

TABLE 1. COMPARISON OF THE AFTER EFFECT OF EXPOSURE OF NORMAL AND ADRENALECTOMIZED ALBINO RATS TO COLD (4°C.) MEASURED AT 29°C.

Rat	Duration of exposure hours	Heat production in cal. per sq. m. per 24 hr.								% change in basal after adrenalectomy
		Normal			Double adrenalectomized					
		Before	After	Change %	Day of test	Survival days	Before	After	Change %	
5A	2	878	894	+ 2	3	6	806	804	− 0	− 8
3	2	883	1226	+39	5	8	844	839	− 1	− 4
4	2	927	1230	+33	5	7	793	727	− 8	−15
2	2	914	1038	+14	5	6	935	536	−43	+ 2
1	2	946	1077	+14	5	6	825	447	−46	−13
5	2	833	989	+19	5	14	742	632	−15	−11
6	2	845	889	+ 6	5	12	810			− 4
7	1	817	973	+19	5		652	599	− 8	−20
					7	9	624			−24
9	1	858	1020	+19	7	9	772	564	−27	−10
8	1	680	865	+27	7	9	695	720	+ 4	+ 2
10[1]	1	832	959	+15	5		791	696	−12	− 5
					7	10	647	672	− 4	−23
12[1]	1	830	1147	+38	2		909	1078	−37	+10
					5	5	733	570	−22	−12
8A	2	917			4	7	590			−40
7A	2	830			5	14	817			− 1
					9		803			− 3
Average		856	1026	+20			765	683	−11	−10

[1] Small amount of accessory tissue.

A comparison of the stimulating effects of low temperatures on the heat production of normal and adrenalectomized rats showed that in every case animals with none or very little adrenal tissue gave less response than did normals. The average difference between the stimulating effect of cold in

normal and adrenalectomized animals was 33 per cent. This was twice the difference reported (1) following removal of a single adrenal.

Immediate effect of cold. When normal rats were exposed to a temperature of 4°C., they responded by a greatly increased heat production. This effect seemed to be independent of shivering. The calory output varied from 2100 to 2500 cal. per square meter per 24 hr. The previous basal determinations for these animals averaged 833 cal. per square meter per 24 hr. The mean increase in the 8 rats studied was 176 per cent, the range being from 227

TABLE 2. COMPARISON OF HEAT PRODUCTION IN NORMAL AND DOUBLY ADRENALECTOMIZED ALBINO RATS AT 29° AND 4°C.

Rat	Exposure to cold hours	Heat production in cal. per sq. m. per 24 hr.							
		Normal			Doubly adrenalectomized				
		At 29°C.	At 4°C.	% change	Day of test	Survival	At 29°C.	At 4°C.	% Change
7A	2	829	2201	+167	5		817	1638	+100
					9	14	803	1437	+ 79
5A	2	878	2392	+172	3	6	806	1371	+ 70
3	2	883	2514	+185	5	8	844	1936	+129
8A	2	917	2123	+132	4	7	590	1162	+ 97
7	1	817	2434	+198	5		652	957	+ 47
					7	9	624	655	+ 5
8	1	680	2225	+227	7	9	695	1279	+ 84
10[1]	1	832	2198	+188	5		791	1349	+ 69
					7	10	647	1384	+114
12[1]	1	830	2278	+175	2		908	1641	+ 81
					5	5	733	1057	+ 44
Average		833	2296	+176			743	1322	+ 79

[1] Small amount of accessory tissue.

to 131 per cent (table 2). Control tests indicated that the operation itself produced no effect on the heat production in cold except to increase the metabolism to a slight degree.

When rats had both adrenals removed, and their heat production measured at 4°C., there was a remarkable decrease in the number of calories produced. The colory output varied from 650 to 1940 cal. per square meter per 24 hr. Ignoring the very lowest value, the mean increase was 79 per cent (range of 44 to 129 per cent). The loss of both adrenals resulted in an average decrease of 96 per cent in the metabolic response to cold. This was more than 3 times the difference previously reported following the removal of one adrenal.

From the data thus far available, there seems to be a rough quantitative relationship between the amount of available adrenal tissue and the extent of the metabolic response of the animal to cold. Whether such a relationship would hold for other species besides the rat is yet to be determined, and offers a fruitful field for further investigation.

Body temperature. Adrenalectomized animals are unable to maintain normal body temperature on prolonged exposure to cold, but a marked fall in body temperature was not observed until after 2 hours in the cold (*3, 4*). The colonic temperatures of 14 young, normal rats (fig. 1) at the end of the second hour averaged 38.1°C., and ranged from 37.6 to 38.8°C. After adrenalectomy and at 4°C., the calory temperatures for the same time averaged 36.9°C., and ranged from 36.7 to 37.1°C. The curve shows the rather

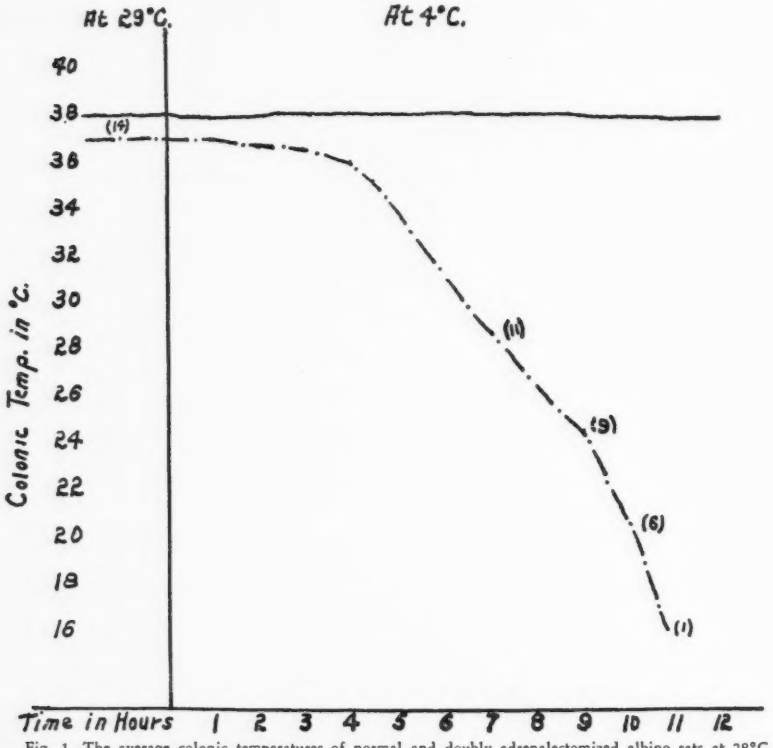

Fig. 1. The average colonic temperatures of normal and doubly adrenalectomized albino rats at 28°C. followed by exposure to a temperature of 4°C.
Solid lines = normal animals. Broken lines = adrenalectomized animals. Figures in parenthesis indicate number of survivors at the time.

rapid and sudden failure of the heat regulating mechanism of adrenalectomized animals placed under the stress of cold. These animals were able to maintain their temperatures almost up to their values at 28°C. for some hours before failure occurred. The first of these rats died at the end of the 7th hour, and all had died by the 12th. The lowest body temperature recorded was 16.4°C. None of the normal rats had succumbed even after a 24-hour or longer period of exposure.

Upon exposure to cold, adrenalectomized animals may produce the extra heat required for a short time, but this finally fails so that the body temperature

drops. Reflex activity is involved in the reaction to cold. It is possible that eventually the failure of the body musculature to produce more heat is due in part to fatigue of these reflexes, as well as to depleted carbohydrate stores or to changes in the heat-producing tissues themselves. Whatever the ultimate cause of the failure of the heat regulating mechanism, it seems that the adrenals provide the guiding factor in this adjustment. The fall in metabolism seems to be a real effect of adrenal insufficiency. Ring (2) and also Baird et al (3) have shown that the thyroid played little or no part in such a response. Removal of the thyroid did not affect the metabolic response to cold. Baird (3) presented evidence that the loss of resistance to cold temperatures seen in hypophysectomized rats was due, in part at least, to an associated cortical insufficiency and could be corrected by injections of cortin. Cortin injections (4) also enabled adrenalectomized rats to raise their body temperatures to normal levels and to produce heat as well as normals. Injections of adrenalin were ineffective in restoring the resistance to cold of adrenalectomized or hypophysectomized rats. If, as it seems, the lowered resistance to cold is due to adrenal insufficiency, the measure of the metabolic response to cold may prove to be of some aid in the diagnosis of Addison's disease. That this may be a valuable test is indicated by the fact that the expected increased heat production response to cold is invariably decreased not only in animals with both adrenals removed, but also in those lacking a single adrenal. Several studies (5, 6) on the increased metabolic response of normal individuals on exposure to temperatures as low as 2°C. have been reported in the literature. Thus normal standards are available for comparative studies on persons having Addison's disease.

SUMMARY

Studies of the respiratory metabolism of normal and completely adrenalectomized albino rats at environmental temperatures of 29° and 4°C., and at 29°C. after exposure to 4°C. for periods of 1 to 2 hours show that the basal heat production of albino rats is lowered approximately 10 per cent following bilateral adrenalectomy. No stimulation of the heat production measured at 29°C. was observed in adrenalectomized rats exposed to a temperature of 4°C. There was a decrease of 10.6 per cent in comparison to an increase of about 20 per cent in the normals. At an environmental temperature of 4°C., the heat production was increased 79 per cent in the adrenalectomized animals compared to the 176 per cent increase observed in normal rats. Adrenalectomized rats were able to maintain postoperative colonic temperatures for several hours, but failure occurred rapidly thereafter. This fall was due to a decreased heat production.

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ARE THE LACTOGENIC AND CARBOHYDRATE METABOLISM HORMONES IDENTICAL?[1]

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Lactation requires the mobilization of considerable amounts of carbohydrates (glucose and lactic acid) in the blood for the formation of lactose by the mammary gland (1). Following the discovery of the lactogenic hormone (lactogen), as well as a carbohydrate metabolism factor in the pituitary, the question arose as to whether these factors are identical or are separate principles. Evans (2) observed hyperglycemia and glycosuria in dogs injected with extracts rich in lactogen, but attempts to separate the two factors were unsuccessful. Nelson, Turner, and Overholser (3) injected monkeys and rabbits with potent lactogenic preparations. In the monkeys, lactation was not induced, neither was the blood sugar level elevated nor glycosuria observed. In pseudopregnant rabbits, marked lactation was induced, but no significant alteration in the blood glucose level occurred. Young (4) failed to secure an elevation of blood sugar or glucosuria in fasting rabbits by the injection of the lactogenic hormone. In a later brief report, Young (5) claimed to have evidence to show that the 'glycotropic' factor was not identical with the thyrotropic, gonadotropic or lactogenic factors. Russell (6) recently described an assay method for a 'glycostatic' factor of the pituitary which maintained muscle glycogen in fasted, hypophysectomized rats. She concluded that glycostatic activity was not associated with any of the following established anterior lobe hormones: growth, adrenotropic, gonadotropic, lactogenic, or thyrotropic. Bennett (7) presented evidence indicating that the glycostatic and the adrenotropic factors are not identical.

In contrast to the mammalian studies are those of Riddle, et al (8) with pigeons and doves. They reported that prolactin, practically free of FSH and thyrotropic hormones, in doses of 5 to 30 u daily for 1 to 8 days, increased the blood sugar in all groups tested by an average of 20 per cent in samples drawn 18 to 25 hours after the last injection. On the other hand, pituitary extracts free of prolactin but thought to contain probably effective amounts of all other anterior pituitary hormones, had little or no effect on the blood sugar of the birds. They concluded that in pigeons the diabetogenic response is a response to lactogen.

In our laboratory, indirect evidence for the individuality of these two

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pituitary effects came from the study of the maintenance of lactation in hypophysectomized guinea pigs. It was found possible to maintain lactation only when in addition to the lactogenic and adrenal cortical or adrenotropic hormones frequent injections of glucose were administered to maintain the blood sugar. Even excessive amounts of lactogen failed to maintain the sugar level (9).

The object of the present paper is to describe a method for the separation of the anterior pituitary into two fractions, one of which is rich in the lactogenic hormone and poor in the carbohydrate metabolism hormone and the second rich in the carbohydrate factor and poor in the lactogenic hormone, thus furnishing additional evidence for the separate identity and individuality of the two pituitary stimulated effects.

METHODS AND RESULTS

Assay of the lactogenic and carbohydrate metabolism hormones. For the assay of the lactogenic hormone, the bioassay method of McShan and Turner

TABLE 1. ASSAY OF THE EXTRACTS FOR THE LACTOGENIC HORMONE

Pituitary Extract	Pigeons Used	Level of Extract Injected (mg.)	Pigeons Showing Crop Proliferation (%)	McShan-Turner U/gm.	Relative Lactogenic Hormone Concentration
Lactogenic	20	0.15	45	6666	100.00
	20	0.20	70		
Carbohydrate metabolism	20	8.0	50	125	1.875
	10	10.0	70		
	20	15.0	95		

(10) was used. They defined a pigeon unit of lactogen as "the total amount of hormone which, injected during a period of four days, will cause a minimum but definite proliferation of the crop glands of 50 $\pm$ 11 per cent of 20 common pigeons weighing 300 $\pm$ 40 gms." For the assay of the carbohydrate metabolism hormone the method described by Bergman and Turner (11) has been used. The guinea-pig unit of this hormone is defined as "the minimum amount of extract, which injected intraperitoneally into well nourished male guinea pigs weighing from 180 to 220 gms., will cause after eight hours an average increase of 50 per cent (to about 165 mg. per cent) in the blood sugar of five or more animals."

Method of extraction and separation. The method of extraction to be described is quite similar to the method of preparation of the lactogenic hormone suggested by Bates and Riddle (12) and shown by Bergman and Turner (13) to be superior to other proposed methods of extraction. As a number of alternative methods have been suggested by Bates and Riddle, it seems important to describe briefly the exact procedure followed in our work.

Acetone dried anterior pituitary powder (from sheep or cattle) is extracted 4 successive times with 15 to 20 times its weight of 60 per cent alcohol at a pH of between 9 and 10 (with NaOH). The coarse residue is then separated

with a bottle centrifuge and the fine residue removed with a Sharples supercentrifuge clarifier. The supernatant fluid from the 4 extractions is combined and the active principles precipitated by adjusting to pH 5.5 to 6 with HCl, followed by the addition of 3 volumes of 95 per cent alcohol. The suspension is held overnight at 0°C. The precipitate is washed with 3 to 4 changes of 95 per cent alcohol, twice with ether, and then dried in a current of warm air. The dried material is then ground and run through an 80 mesh sieve. This fraction is referred to as the *initial extract*.

Assay of the initial extract of sheep powder showed that it contained about 250 guinea-pig units of the carbohydrate factor (11) and 3700 pigeon units of the lactogenic hormone per gram.

The lactogenic hormone is then separated by extracting 20 gm. of the initial extract with one liter of water at pH 9.5. The solution is adjusted to pH 5.5 to 6 with H_2SO_4. Then Na_2SO_4 is added to bring the solution to a concentration of 0.5 per cent. The solution is then brought to pH 3.5. The precipitate is removed in the supercentrifuge. The above extraction is repeated twice. The precipitate contains the lactogenic hormone. The further separation of the supernatant fluid (*I*) will be described below.

The lactogen fraction is further purified by extraction with one liter of 70 per cent alcohol at pH 9. NaCl to a concentration of 0.15 per cent is then added, the solution adjusted to pH 6, and the precipitate removed in a supercentrifuge. This extraction is repeated 2 or 3 times. The precipitate contains very little lactogen. The supernatant fluids are combined, adjusted to pH 7 and concentrated in a vacuum below 37°C. until the alcohol is removed. The suspension is then adjusted to pH 4 to 5, centrifuged, washed with alcohol and ether, and dried. This fraction is rich in the lactogenic hormone.

The supernatant fluids (*I*) are combined and adjusted to pH 4.5 with NaOH. Flavianic acid to a concentration of 0.1 per cent is added and held overnight at 0°C., the precipitate is removed in a centrifuge, washed with alcohol and ether, dried, and ground. The extract may be decolorized by suspending 1 gram of the powder in 100 cc. of 80 per cent alcohol to which 1 per cent NH_4OH has been added. After standing overnight at 0°C., the precipitate is recovered, washed in alcohol and ether, dried, and ground to a powder. The nearly colorless preparation is rich in the carbohydrate metabolism hormone. Incidentally, it is also rich in the thyrotropic and gonadotropic hormones. Work is now in progress in which an attempt is being made to separate these several hormones.

Results of biological assay. The extracts containing the lactogenic and carbohydrate metabolism factors have been carefully assayed to determine the amounts of these two factors which each contained. The lactogenic fraction gave a unit response by the McShan-Turner method with 0.15 mg. (table 1). A further test at a 0.20 mg. level showed a 70 per cent pigeon response, which is a good check according to their assay curve. One gram of this preparation contains about 6666 pigeon units of lactogen. The presence of the carbohydrate hormone was tested at levels of 20, 40, and 100 mg. (table 2). It was found that 100 mg. were required to equal a guinea pig unit, according

to the method of Bergman and Turner. In other words, one gram of the lactogenic fraction contained 10 u of the carbohydrate metabolism hormone.

The carbohydrate metabolism hormone fraction, when assayed for its content of lactogenic hormone, showed a unit response when 8 mg. were injected. Further assays at 10 and 15 mg. levels showed 70 and 95 per cent pigeon responses which check well with the assay curve. One gram of the carbohydrate hormone fraction contained 125 pigeon units of lactogen as a contaminant. The assay of the carbohydrate metabolism hormone was made at levels of 1.5, 2, 4, and 5 mg. One unit of hormone appears to be contained

TABLE 2. ASSAY OF EXTRACTS FOR CARBOHYDRATE METABOLISM HORMONE

Pituitary Extract	Number Guinea-pigs Used	Level of Extract Injection (mg.)	Individual Guinea Pig Response, Blood Sugar (mg. %)	Average Blood Sugar (mg. %)	Bergman-Turner U/gm.	Relative Carbohydrate Hormone Concentration
Carbohydrate metabolism	10	1.5	127, 153, 166, 141, 151 111, 125, 153, 192, 203	152.2		
Carbohydrate metabolism	10	2.0	181, 170, 178, 199, 176 135, 164, 211, 221, 189	182.4	500	100
	6	4.0	227, 190, 215 213, 201, 212	209.7		
	8	5.0	185, 259, 243, 229 202, 212, 237, 261	227.0		
Lactogenic	10	20.0	128, 109, 128, 114, 127 101, 140, 102, 114, 112	117.5		
	10	40.0	146, 168, 105, 148, 135 113, 160, 158, 162, 118	141.3		
	10	100.0	148, 195, 202, 170, 139 225, 180, 143, 120, 161	168.3	10	2

in slightly less than 2 mg. At the 2 mg. level, one gram of this extract would contain 500 guinea pig units.

From these data it appears that the lactogenic and carbohydrate metabolism hormones are not identical; in fact, the evidence presented would indicate that it is possible to make a separation of these two factors, having ratios of activity of about 1:50, respectively.

DISCUSSION

Two schools of thought exist as to the number of different factors which are secreted by the anterior pituitary. Some workers try to minimize the number of separate factors, accepting only the gonadotropic, thyrotropic, and lactogenic. Others may be called 'splitters' and find a large number of separate factors including two gonadotropic, thyrotropic, lactogenic, parathyrotropic, adrenotropic, pancreatropic (islets), mammogenic (growth of mammary gland), carbohydrate metabolism, fat metabolism, and growth or nitrogen metabolism, etc. While not all of these effects have been proven conclusively, the principal point of difference rests upon the individuality or

duality of the compounds which cause these effects. Thus the lactogenic factor which initiates and maintains secretory activity of the mammary glands has been thought to be identical with the carbohydrate metabolism and adrenotropic factors, all of which are important in the milk secretion process.

Two types of evidence favoring the individuality of these factors may be obtained. The assay of various pituitary preparations and extracts may show distinctly different ratios of content of the various factors which would not be true if they were identical compounds. The second method consists in the chemical separation of the various factors. Because of the probable chemical similarity of a number of these factors, the latter method will probably require time to develop.

In the present study, however, it has been found possible to make a rather complete chemical separation of the lactogenic and the carbohydrate metabolism factors. The amount of contamination of each factor by the other may be expressed by rations of about 1:50, respectively. Therefore, these two pituitary factors appear to be separate and distinct hormones as indicated by chemical separation and biological assay.

SUMMARY

A method is described for the separation of two fractions of the anterior pituitary, one of which is rich in the lactogenic and the other in the carbohydrate metabolism hormone. By biological assay methods it was shown that the lactogenic fraction contained about 6666 pigeon units per gram of lactogen by the McShan-Turner method; whereas the carbohydrate metabolism fraction contained only 125 pigeon units per gram, or less than 2 per cent as much lactogenic hormone activity. The carbohydrate metabolism fraction was observed to contain about 500 guinea pig units of the carbohydrate metabolism hormone per gram by the Bergman-Turner method; whereas the lactogenic hormone contained only 10 guinea pig units per gram, or about 2 per cent as much carbohydrate metabolism activity. The separate identity of these two pituitary stimulated phenomena are thus indicated by chemical separation and by biological assay.

The separation and purification of the two fractions described has increased the potency of each extract by about 100 per cent over the assay of the initial extract.

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FATTY ATROPHY FOLLOWING INSULIN INJECTION IN NONDIABETIC MALNUTRITION

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The use of insulin to produce a gain in weight in certain cases of nondiabetic malnutrition has proved of considerable value in 100 cases (1) observed from 1 to 6 years after discontinuing insulin. The added weight in these cases was due to an actual increase in the fat deposit which was demonstrated in one case (2) with biopsies taken from the abdominal wall before and after insulin therapy. However, it seemed somewhat paradoxical to find that fatty atrophy occurred in one case at the site of insulin injections in spite of a general increase in the fat deposit.

In the abundant literature which has accumulated on the use of insulin in malnutrition no observations have been reported on the occurrence of fatty atrophy following the use of this therapy, although it has been noted occasionally in diabetes mellitus. This appeared to be an interesting phase of the subject and this paper, therefore, reports some observations on the local atrophy which occurred in one patient with nondiabetic malnutrition at the site of insulin injections about 4 or 5 months after the omission of the insulin.

Insulin atrophy in diabetes. Local atrophy has been observed to occur in patients with diabetes mellitus at or near the site where various doses of insulin had been administered most frequently for 3 to 42 months. Barborka (3) and Depisch (4) were the first to describe this condition. Among others to report cases later were Joslin (5), Chapman (6), McCormick and Moorhead (7), Eeg-Olofsson (8), Boller (9), Rowe and Garrison (10), Engelbach (11), Schlüter (12) and Rosenberg and Berliner (13).

The lesions were characterized by a disappearance of the subcutaneous fat which resulted in depressions in the skin varying in diameter from 2 to 9 cm. and in depth from 1 to 3 cm. These were usually permanent, although in rare instances there was a return to normal. They were not associated ordinarily with any particular pain or infection from the injections, or with any neurological changes. The atrophy appeared much more commonly in females and youthful diabetics than in males and, according to Joslin, exceptionally in those whose diabetes was controlled.

Although the cause is unknown, several etiologic factors have been suggested. Carmichael and Graham (14) thought that repeated injections of insulin in one localized area resulted in changes in the subcutaneous fat presumably due to a lipolytic ferment, since insulin is prepared from the pancreas. However, Rabinowitch (15) was unable to confirm this impression when

he concentrated large volumes of insulin and was unable to detect any lipase. Fischer (16) believed the atrophy was due to a low grade inflamation and Eeg-Olofsson (8) found on microscopic examination areas of round cell infiltration similar to that occurring after any hypodermic injection. On the contrary, Mentzer and Dubray (17) and Price (18) were unable to find any lymphocytic foci in the depressed areas indicative of inflammatory changes. Lawrence (19) observed that insulin injections produced a temporary edematous exudate which tended to slight fibrosis in the subcutaneous fat. Avery (20) felt that the fat atrophy was a traumatic atrophic panniculitis due to repeated small traumata to the panniculus adiposis from many injections of insulin. Davison (21) reported the case of a patient with local sensitivity to insulin who developed atrophy after 4 months of insulin injections, whereas Carmichael and Graham (14) found no association of atrophy with local reactions. Williams (22) thought that insulin possibly injured the membrane which covered the fat cells and released fat globules which acted as foreign bodies and resulted in the formation of histiocytes which took on a lipophagic activity. Nichols (23) believed that the strong concentration of insulin near the site of injection caused a local oxidation of carbohydrate, which in turn resulted in the combustion of local fat.

Insulin atrophy in nondiabetic malnutrition. A girl, aged 22 years, was seen first November 5, 1934, compaining of underweight. Her family history showed that her father was very ill with malignancy of the larynx. In the past she had always been quite well, except for influenza in 1918. However, she was very allergic to strawberries, wool, fish, timothy and ragweed. The menses were normal. After graduating from college in 1933 she weighed 111 lb., but during the ensuing months she lost 13 lb. and did not feel well.

The physical examination gave essentially negative findings except for undernutrition. Her height was 65 inches, her weight 98 lb. Blood and urine tests were normal.

She was given a high calorie diet and proper instructions for insulin administration. From November 6 to December 1 she injected herself with 10 U of insulin subcutaneously 3 times a day, about 20 minutes before meals. As a result she gained 13 lb. and felt better in spite of the nervous strain of her father's illness. After omitting insulin she put on an additional 4 or 5 lb. during the month, and this she has retained. Ten days after starting insulin she developed a local sensitivity to it, which persisted during the period of therapy.

About 4 or 5 months after the withdrawal of insulin the patient noted a dimple in the right thigh at the site of the injections, which were not associated with any soreness or infection. Subsequently this increased in size and reached its maximum in about another 4 months. During the past 2½ years the size has remained stationary except during pregnancy. The patient was married on November 1, 1935 and gave birth to a child on January 2, 1937. During pregnancy she gained 20 lb. and noted that the depression on the thigh was more marked, since there was an increased deposit of fat in the thighs but not in the atrophic area. After delivery, her weight dropped to 119 lb., her present weight, and the atrophy became less manifest and about as obvious as before pregnancy.

Examination of the right thigh now shows an oval depression on the anterior aspect with a diameter of 6 cm. and a depth of 0.9 cm. The sensation to pin-prick

in this area is definitely diminished, as also to a less extent in a band 1 cm. in width surrounding the circumference of the depression.

In going into the history of the insulin administration, it was found that the patient always gave the morning and evening injections in the right thigh and the noon injection in the left thigh. Consequently, there were twice as many injections in the right thigh as in the left thigh.

COMMENT

This patient is of unusual interest because she is a nondiabetic thin person who took insulin for only 3 weeks to gain weight, and 4 or 5 months after omitting insulin the onset of local atrophy was noted in the right thigh where the drug was administered twice as frequently as in the left thigh. This was in striking contrast to the diabetics who developed local atrophy after the continuous use of insulin for a number of months. However, it is difficult to know whether it required so many months of insulin injections to produce the atrophy, or whether the atrophy would have occurred so many months after only a few injections, even though the insulin might have been stopped in the diabetics.

It is interesting to speculate as to what was going on in the subcutaneous tissues at the site of the atrophy in this patient during the months after the insulin was omitted until the depression was noted. It seems likely that the pathological process of the fat atrophy began during the period of insulin administration, and continued slowly during the ensuing months, finally becoming sufficiently marked to produce a noticeable depression. This was probably associated with a nervous disturbance, since there was a decreased sensation in the region of the atrophy.

Just why a patient of this type should develop fat atrophy under insulin treatment is conjectural. There is no reason to believe that the cause is any different in the nondiabetic than in the diabetic. Nevertheless, it does show that a patient does not have to be diabetic or fat to develop insulin atrophy, and that the atrophy may appear some months after the withdrawal of insulin. Local sensitivity is probably not a factor in the causation of this atrophy even though this patient was allergic, because 35 of the author's nondiabetics with malnutrition, 2 with asthma and 2 with hay fever, had local reactions to insulin, and no other individual developed the atrophy. It appears that there is some individual susceptibility to this lesion which is common to females and children, and not especially to males, in whom the condition is so rare.

SUMMARY

This paper presents some observations on local fat atrophy which occurred in a normal, thin person who took insulin for 3 weeks to gain weight. About 4 or 5 months after the insulin was omitted, the atrophy was noted in the right thigh where the drug was injected twice as frequently as in the left thigh. This increased in size and reached its maximum diameter of 6 cm. and depth of 0.9 cm. in the subsequent 4 months. During the past 2½ years the depression in the thigh has remained stationary except during pregnancy, when it appeared more marked. The sensation is diminished over the region

of the atrophy. This case illustrates that a patient does not have to be diabetic or fat to develop insulin atrophy, and that the atrophy may appear some months after the withdrawal of the insulin.

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CLINICAL AND LABORATORY NOTES

INFLUENCE OF ADRENAL CORTEX EXTRACT UPON COMPENSATORY HYPERTROPHY OF THE ADRENAL CORTEX

EATON M. MACKAY AND LOIS LOCKARD MACKAY*

When one adrenal is removed the cortex of the remaining gland undergoes hypertrophy.[1] This does not occur if the hypophysis is removed simultaneously, and in time if hypophysectomy is done, the cortex of the remaining gland, or if the animal has both glands, all of the cortical tissue, atrophies.[2] Cortical repair follows anterior pituitary transplants.[2] An anterior pituitary extract is now available which prevents the adrenal cortex atrophy in the hypophysectomized organism and stimulates the cortical tissue in normal animals.[3] It is fairly obvious that the integrity of the adrenal cortex is, therefore, dependent upon the hypophysis. As far as compensatory hypertrophy of the adrenal cortex is concerned, a reasonable hypothesis is as follows. The maintenance of a normal amount of adrenal cortex tissue is dependent upon the concentration of adrenal cortex hormone circulating in the organism. When one adrenal is removed, the adrenal cortex secretion is reduced and the stimulation received by the other gland from the anterior pituitary becomes increased. A compensatory hypertrophy is the result. If this is true, it might be supposed that an active adrenal cortex extract would reduce or entirely prevent compensatory hypertrophy of the adrenal cortex following unilateral adrenalectomy. Experiments carried out to test this possibility have given a positive result.

Experimental. Our first experiment, which was carried out over 4 years ago with a commercial extract, gave a negative result as was also the case with this same brand of extract more recently. The extract used in this study was a commercial product obtained from the Wilson Laboratories. This was purchased and used more than 2 years ago. Its strength is indicated by data contained in two recent reports.[4, 5]

Our usual strain of inbred albino rats was used. Litter mates through all of the 4 groups comprise experiment *1* and *2*. The operative procedure has been described.[1] In experiments *3* and *4*, since adult animals were used and their adrenals were no longer undergoing changes incident to growth, no unoperated controls were included. The adrenal removed at operation serves as the control. The degree of compensatory hypertrophy is the increase in the weight of the hypertrophied gland above the weight of a normal adrenal expressed as a percentage of the latter.

The details of the various experiments are presented in table 1. In all cases the adrenal cortex extract was administered subcutaneously. The body surface used in adjusting the weights of the adrenal glands for variations in body size is calculated from the body weight by the same formula[6] that we have used before.

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From an inspection of the data on adrenal weights in table 1, it is apparent that the administration of adrenal cortex extract will suppress the compensatory hypertrophy of the adrenal cortex. Histologic evidence, which need not be detailed here, supports this conclusion and shows that the changes are limited to the cortex.

Discussion. Since the completion of this work, several experiments have been reported which are of such a nature that they would lead one to predict the results which have been presented here. Autoplastic or homoplastic transplants of cortical adrenal tissue are successful in rats if all of one adrenal and a good portion of the other gland have been removed.[7] Likewise regeneration from the capsule of an adrenal gland after enucleation is inhibited if one of the glands is left intact.[8] This indicates that regeneration of the adrenal cortex does not occur except when there is a deficiency of secretion of the cortical hormone, and the administration of large amounts of the latter have been found[9] to prevent regeneration of the cortex of the adrenal gland after enucleation even when the other gland has been removed.

TABLE 1

	1	2	3	4
	Controls	Unilateral Adrenalectomy	Controls—Cortical Extract	Unilateral Adrenalectomy—Cortical Extract
	Experiment 1			
	Female rats 45 days old when operated upon. Killed 10 days later. Each rat in groups *3* and *4* was given 0.5 cc. adrenal cortex extract twice daily during this period.			
No. rats	7	7	7	7
Body weight—gm.	99	99	93	98
Body surface—sq. cm.	242	242	233	241
Adrenal tissue—mg.				
Max.	25.0	38.0	11.5	19.0
Min.	13.5	23.5	19.5	23.0
Av.	36.3	31.1	30.2	21.0
Av./dcm. B.S.	15.0	12.9	12.9	8.7
Compensatory hypertrophy—%		72		16
	Experiment 2			
	Female rats 75 days old when operated upon. Killed 8 days later. Each rat in groups *3* and *4* was given 0.5 cc. of adrenal cortex extract daily the first 3 days and 1.0 cc. per day the last 5 days.			
No. rats	10	10	10	10
Body weight—gm.	143	140	141	136
Adrenal tissue—mg.	309	305	306	301
Max.	41.0	26.7	34.4	16.9
Min.	20.1	13.4	20.4	8.1
Av.	31.4	20.0	24.9	14.4
	10.2	6.5	8.2	4.8
Av./dcm. B.S.				
Compensatory hypertrophy—%	73			0

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TABLE 1. (*Continued*)

	1	2	3	4
	Controls	Unilateral Adrenalectomy	Controls—Cortical Extract	Unilateral Adrenalectomy—Cortical Extract
Experiment 3				
Female rats of approximately the same age and weight which were operated upon and sacrificed 8 days later. During 4 days of this period group *4* received 0.5 cc. and during the last 4 days 1.0 cc. of adrenal cortex extract each day.				
No. rats		7		7
Body weight—gm.		204		204
Body surface—sq. cm.		394		394
Adrenal tissue—removed at operation—mg.				
Min.		12.8		14.4
Max.		23.6		22.5
Av.		19.3		18.1
Av./dcm. B.S.		4.9		4.6
Adrenal tissue after compensatory hypertrophy—mg.				
Min.		22.4		15.8
Max.		41.4		25.5
Av.		31.8		19.9
Av./dcm. B.S.		8.1		5.0
Compensatory hypertrophy—%		65		9
Experiment 4				
Male rats of nearly the same age and weight treated as those in Experiment *3*.				
No. rats		4		4
Body weight—gm.		280		275
Body surface—sq. cm.		484		479
Adrenal tissue removed at operation—mg.				
Min.		11.0		11.1
Max.		20.5		21.1
Av.		14.7		14.4
Av./dcm. B.X.		3.0		3.0
Adrenal tissue after compensatory hypertrophy—mg.				
Min.		19.2		13.3
Max.		31.1		16.7
Av.		24.4		15.7
Av./dcm. B.S.		5.0		3.2
Compensatory hypertrophy—%		67		7

Now it has been shown[10] that atrophy of the adrenal cortex can be produced in the normal rat by the administration of large amounts of adrenal cortex extract, but that this atrophy may be prevented by the simultaneous administration of the adrenotropic fraction of anterior pituitary extract. The atrophy due to adrenal cortex extract administration is exhibited in a minor degree by the extract-treated control groups in the present study (group *1* and *3* in experiments *1* and *2*).

The relationship of the thyroid and of the adrenal cortex to the anterior pituitary is very similar.[10] Consequently it is probable that the mechanism by which the thyroid hormone prevents the compensatory hypertrophy of the thyroid gland[11] is similar to that involved in the suppression of compensatory hypertrophy of the adrenal cortex by adrenal cortex extract.

Summary. When one adrenal gland is removed the cortex of the remaining gland undergoes a compensatory hypertrophy. This compensatory hypertrophy is suppressed or prevented by the administration of adrenal cortex extract. It is probable that the hypertrophy is dependent upon stimulation by an anterior pituitary hormone, the production of which depends on the concentration of adrenal cortex hormone circulating in the organism. When the lack of this hormone follows unilateral adrenalectomy, compensatory hypertrophy does not ensue when the stimulation by the anterior pituitary is prevented by supplying the deficiency in cortical hormone from outside sources.

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FAILURE OF ATROPINE TO PREVENT OVULATION FOLLOWING COITUS IN THE RABBIT

A. W. MAKEPEACE*

That atropine, given intravenously in doses of 2.5 mg./kg., would prevent ovulation normally occurring in rabbits after coitus was reported by Foster, Haney and Hisaw.[1] An attempt was made to repeat this work before making use of the phenomenon described. It was found in a few rabbits that ovulation was not inhibited by atropine in the doses given. A more comprehensive experiment was therefore planned and the results are now presented.

Methods. Three samples of atropine sulphate were used, from 2 different drug firms. All samples were of U.S.P. grade, 2 were granular and 1 powdered. The drug was injected in aqueous solution into the marginal ear vein of the rabbit. A total of 12 animals were given varying doses of atropine and accepted the buck at varying intervals after injection. The details of the various experiments are shown in table 1.

Discussion. Very little need be added to the evidence as presented in table 1. Atropine in doses ranging from 1.5 to 6.0 mg./kg. did not inhibit ovulation. All but 2 of the animals accepting coitus, when opened 24 hr. after mating, exhibited normal freshly ruptured follicles in the ovaries. From a survey of the past history of the 2 animals which did not ovulate, it was found that they did not measure up to a good standard for healthy estrous rabbits. That animal receiving 3.0 mg./kg. and which did not ovulate had had a distinctly abnormal delivery, i.e., dead, 5-day postmature fetuses. The animal receiving 6.0 mg./kg. and not ovulating was 6 weeks postpartum, and also had been used for another experiment which necessitated opening the abdomen of the rabbit during this interval. All of the other animals were healthy postpartum or known postpseudopregnant rabbits. In addition to the 3 animals which received 6 mg./kg. of atropine and accepted the buck, 11 other healthy postpartum or postpseudopregnant animals were injected at this dose level and refused copulation. These does were either not in heat or were thrown out of heat by atropine. It is our opinion that in the great majority of cases the latter explanation is the true one.

Hammond and Marshall[2] state that, when using previously isolated rabbits, 35 per cent of consummated matings will be infertile over a 12-month period. Friedman[3] working with isolated stock and giving atropine shortly after coitus found that 7 out of 10 rabbits failed to ovulate. In contradistinction to these 10 animals, he found that 5 postpartum rabbits treated identically had, in each case, ovulated. Hammond and Marshall[2] state that healthy postpartum rabbits will invariably accept the buck and will (in the breeding season) always ovulate.† And finally, with the exception of the 2 substandard animals noted above, all of our

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† Our experiments were carried out during the spring months, which constitute the optimal breeding period of the year.

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postpartum animals accepting coitus ovulated regardless of the dose of atropine.

We have not worked with isolated animals because of the inconstancy of post-coital ovulation. To demonstrate any inhibition of ovulation in this type of animal, one would have to resort to a large number of observations with a statistical treat-

TABLE 1

Rabbits	Dose of Atropine	Interval Between Injection and Mating	Result	
Number	mg./kg.	Minutes	Ovulation	
			+	−
2	1.5	11±1	2	0
3	3.0	10±1	2	1
1	3.0	15±1	1	0
3	3.0	19±1	3	0
2	6.0	15	1	1
1	6.0	22	1	0

ment of the incidence of ovulation with and without atropine. In our hands, however, the postpartum animal will ovulate following coitus and will do so even though a dose of 6 mg./kg. of atropine is injected before mating.

Summary. Atropine sulphate given intravenously in doses up to 6.0 mg./kg. to healthy postpartum (estrous) rabbits does not prevent ovulation following coitus. Atropine in doses of 6.0 mg./kg. throws many estrous rabbits out of heat.

THE EFFECT OF TESTOSTERONE PROPIONATE ON HUMAN FEMALE CASTRATES*

CHARLES H. BIRNBERG, LAWRENCE KURZROK, AND SEYMOUR LIVINGSTON

The severe menopausal symptoms resulting from surgical castration of the female are familiar to all. The administration of estrone† in various amounts, depending upon the patient's response to treatment, is a most satisfactory method for the control of these menopausal symptoms. However, the possibility of reactivating the endometrium is always a factor to be considered, and it was because of two such cases that other methods for relief of symptoms were sought.

Theoretically, if large doses of estrone can depress the pituitary and in this manner relieve menopausal symptoms, why can not large doses of male sex hormone act similarly, and without fear of activating the endometrium? Working upon this hypothesis, large doses of testosterone propionate were administered to 3 women who had previously been castrated by radium or x-ray.

Case 1. Mrs. J. P., aged 46 years, had received two years previously a castration dose of x-ray for very profuse vaginal bleeding. Menopausal symptoms such as flushes, sweats, headaches and irritability appeared almost immediately and became progressively worse until the patient had a flush followed by a sweat almost every 5 minutes. Attempts to control these symptoms with small doses of estrone failed. About a year ago the patient was given 50,000 I.U. of estrone 3 times a week for 6 weeks. She was greatly improved, and the dose was reduced so that she was comfortable without any flushes upon receiving 5000 I.U. once in 3 weeks. After 8 months of therapy the patient began to bleed, and an endometrial biopsy performed at that time revealed active proliferative endometrium. Estrone therapy was immediately discontinued, and after 4 weeks her distressing symptoms recurred. At this time she was given 10 mg. of testosterone propionate twice a week; after 3 weeks of therapy her symptoms subsided, and now she can be maintained on 10 mg. every 2 weeks. An endometrial biopsy performed 3 months after therapy was started revealed an atrophic resting endometrium.

Case 2. Mrs. E. D., aged 40 years, had received a castrating dose of radium 6 months previously. Menopausal symptoms such as flushes, headaches, extreme nervousness and irritability set in almost immediately. She was given 10 mg. of testosterone propionate twice a week for 6 weeks, and then 10 mg. once a week for 6 weeks. She now receives 10 mg. once every 12 days and is symptom-free.

Case 3. Mrs. M. W., aged 48 years, had received radium for severe vaginal bleeding 6 years previously. Menopausal symptoms did not appear until 2 years later, and at the beginning were controlled with small doses of estrone. However, her symptoms became worse and it was necessary to increase the dose of estrone, so that finally her symptoms were under control only when she received 50,000 I.U. every 2 weeks. However, vaginal bleeding reappeared after several months' treatment with these large doses of estrone. An endometrial biopsy revealed the presence of

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† We are greatly indebted to Dr. Stragnell, Dr. Schwenk and Dr. Gilbert of the Schering Corporation for their assistance and for the liberal supply of oreton and progynon-B which made this work possible.

interval endometrium. Treatment was then discontinued, but with the recurrence of her severe menopause symptoms she was given 10 mg. of testosterone propionate twice a week. Her symptoms disappeared almost immediately and she can now be maintained by giving her 10 mg. of testosterone once every 3 weeks.

Conclusions. Administration of testosterone propionate controlled the severe menopausal symptoms in 3 women castrated by x-ray or radium. There was no re-activation of the endometrium, as proved by endometrial biopsies taken in 2 of the 3 cases. The effect of testosterone propionate on the menopausal symptoms of non-castrated women is now being studied.

CURRENT ENDOCRINE LITERATURE

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BOOK REVIEWS

Biological Standardization. Burn, J. H., Pp. 288, Oxford University Press, New York, London, etc., 1937.

This book is a successor to "Methods of Biological Assay" by the same author. It is an excellent manual of bio-assay, giving adequately detailed directions for the performance of each assay, and is well illustrated by numerous figures. The first 3 chapters deal with fundamentals: standards, classification and mathematical treatment. Procedures for the assay of posterior pituitary, insulin, suprarenal, thyroid, parathyroid, ovarian hormones, male hormones and anterior pituitary are included as well as vitamins, digitalis, strophanthus, squill, ergot and organic arsenic and antimony compounds.

Data on the Growth of Public School Children. Dearborn, Walter F., John W. M. Rothney and Frank K. Shuttleworth, Pp. 136, Society for Research in Child Development, National Research Council, Washington, D.C., 1938.

Endocrinologists who are interested in research on growth and development will find this monograph to be a rich source of carefully obtained primary data. Under the auspices of the Harvard Graduate School of Education, and with the aid of subvention from the Commonwealth Fund, over 1500 children have been measured and tested repeatedly through a 12-year period. The work required the cooperation of many associates, the services of several hundred assistants and the expenditure of thousands of dollars. Several standard anthropometric measurements were taken and indices calculated; the weight, dentition and skeletal age were studied. A series of intelligence tests appropriate to the various ages were administered. As a source of normal control data, the collection is unique.

A Handbook of Methods for the Study of Adolescent Children. Greulich, William W., Harry G. Day, Sander E. Lachman, John B. Wolfe and Frank K. Shuttleworth, Pp. 406, Society for Research in Child Development, National Research Council, Washington, D.C., 1938.

This book represents the combined work of 5 men under appointment as Fellows in Child Study of the General Education Board and who were assigned to the project at Yale University. The study was guided by an advisory committee consisting of Professors Edgar Allen, John Fulton, Mark May, Walter Miles, Grover Powers and

M. C. Winternitz. Supplementary aid was obtained from various members of the Yale faculty and from authorities elsewhere in the different fields that are covered. The book thus becomes an indispensable addition to the libraries of students of human development.

The book treats of such subjects as Anthropometry, genital changes associated with puberty, chemical and physiological aspects, energy metabolism, digestive functions, sensory phenomena, behavior, intelligence, personal and social reactions, hereditary and environmental differences and statistical considerations. The bibliography is extensive and rigidly selected.

The Adolescent Period. A Graphic and Pictorial Atlas. Shuttleworth, Frank K., Pp. 246, Society for Research in Child Development, National Research Council, Washington, D.C., 1938.

As stated in the preface, "this monograph presents samples of the more important facts concerning human adolescents and adolescent development. Although all the charts relate to the second decade of life, the great majority include in addition material on either younger children or adults. Each chart carries a brief but clear explanation, a citation of the original source, and occasionally comments on the nature of the original data. Preference has been given to investigations of American children, to data covering a wide age range, to large numbers of cases, and to recency of publication. Except in a few instances, the content has been taken from the published literature. As far as possible graphic material already available has been utilized in preference to the construction of new charts, but even so nearly two hundred charts represent original drawings prepared especially for this volume."

The charts deal with such subjects as physical growth, development and differentiation; blood pressure and basal metabolism; sexual maturation, physical, physiological and intellectual; health and illness; intelligence; abilities and attitudes; behavioral maladjustments; occupational and sex adjustments and major activities from ages 10 to 24. Boys and girls are treated separately. The material should prove of much use to endocrine investigators who have frequent need of reliable control data.

ADVANCE ABSTRACTS

Changes in the hypophysis and the ovaries of rats chronically treated with an anterior pituitary extract. Collip, J. B., H. Selye and J. E. Williamson. To appear in this Journal.

The experiments reported show that continuous treatment with a pig pituitary extract results in a transient increase in ovarian weight due to the formation of many corpora lutea. This increase reaches its peak more rapidly in the adult rat, but in the immature animal the increase is more pronounced and reaches a much higher level. From this it appears that resistance to such treatment develops more rapidly in the adult than in the immature female. In hypophysectomized rats the peak of the curve is somewhat delayed in comparison with the normal, mature or immature animal, but finally resistance develops even in the absence of the hypophysis, and the ovarian size retrogresses. Parallel with the development of ovarian atrophy 'signet ring' cells appear in the hypophysis, and cysts form in some cases as a result of liquefaction and confluence of these vacuolated sells.

At the height of this acquired resistance, the ovary shows such severe atrophy as is only observed in hypophysectomized animals, and the characteristic 'wheel cells' appear. These ovaries are not only resistant to the gonadotropic action of the extract used but also, at least to some degree, to implants of rat hypophysis. Both these facts indicate that our treatment led to a condition of at least relative immunity to the animal's own hypophyseal secretion. This interpretation received further support by an experiment in which massive doses of a gonadotropic pregnancy urine preparation failed to cause follicle maturation or corpus luteum formation but led exclusively to pronounced theca luteinization. This is otherwise never observed in adult rats treated with such preparations, except after the complete surgical removal of the hypophysis.

An evaluation of the frank method for the determination of urinary prolan in nonpregnant women. Drips, Della G., A. E. Osterberg, Gertrude Fisher and Kathleen Lewis. To appear in this Journal.

An investigation has been made to evaluate the quantitative determination of the urinary prolan in nonpregnant women. The method used for the extraction has been described by Frank, Salmon and Friedman. Albino rats of the Wistar strain were used in the biological assays. The uteri and ovaries were observed grossly and weighed, some at 72 hours, and others at 96 hours, after the first injection. The ovaries were then studied microscopically. The microscopic change in the ovaries at 96 hours appears to be the most reliable criterion for a quantitative estimation of the urinary prolan by this method. On the urine of 248 women 292 determinations were made. Of these 235 presented a variety of clinical conditions, which we thought might be associated with abnormal gonadotropic function of the anterior lobe of the pituitary, and 13 who appeared to be normal were chosen as controls. It is not always possible to demonstrate prolan in the urine of normally menstruating women. We have not found it present in amounts higher than 20 R.U. per liter. When it is present in amounts over 20 R.U., the women may, at any age, present menstrual irregularities and associated clinical symptoms. These include vasomotor disturbances similar to those of the true menopause period during which the values for prolan run as high as 66 R.U. per liter. The degree of severity of the symptoms seems to correspond to the amount of prolan found in the urine.

The effect of hypophysectomy on the restoration of the liver following partial hypophysectomy in rats. Franseen, C. C., A. M. Brues and R. L. Richards. To appear in this Journal.

It was found that the degree of restoration of the liver, after partial hepatectomy, could be calculated with considerable accuracy as percentage of calculated total original liver weight, when the weight of the main liver mass removed at operation was used as the basis of calculation instead of body weight. The restoration of liver tissue after partial surgical removal occurred in the hypophysectomized rats at a somewhat slower rate than in normal rats on an optimum diet, but at a more rapid rate than in fasting rats. Restrained controls restored their livers somewhat better than hyopophysectomized rats. Calculation of the number of cells, and the weight of the liver substance present in the regenerating portion, demonstrated that in both respects the values for the hypophysectomized animals ranged approximately between two-thirds and three-fourths of those for normal animals. This was interpreted as showing that the mass and multiplication of the cells were similarly affected. The percentage of cells undergoing mitosis in the regenerating liver was found to be 0.56 in the hypophysectomized rats and 0.63 in the normal rats at the end of the third postoperative day. Although there appeared to be a slightly lessened ability to restore liver substance after partial hepatectomy among the hypophysectomized animals, as compared with animals not hypophysectomized, it was felt that this could largely be attributed to differences in amount of food ingested by the two groups. The difference was too small to conclude definitely that a direct effect from the absence of the growth-promoting hormone of the pituitary gland was shown upon this example of growth in a parenchymatous organ.

The effect of the administration of thyroxine on the extent of regeneration in the enucleated adrenal of the rat. Ingle, D. J., and G. M. Higgins. To appear in this Journal.

Male rats were treated with thyroxine after enucleation of the left adrenal gland. The effect of thyroxine on the regeneration of cortical tissue was influenced by the presence or absence of the opposite (right) adrenal. When the right adrenal was removed from the body, then the extent of regeneration in the enucleated gland of the thyroxine treated animals exceeded by far the extent in the enucleated glands of the untreated controls. When the right adrenal gland was left intact there were no significant differences between the weights of the enucleated glands of the injected and the control animals, but there was an extensive cortical proliferation in intact glands of the thyroxine-treated group.

The local effect of luteohormone on the uterine mucus of infantile rabbit. Mussio Fournier, J. C., A. S. Albrieux and O. Grosso. To appear in this Journal.

The uterine injection of progesterone brings about a typical change in the mucous membrane of the injected organ. Such a change is more decided than when the same dose of corpus luteum hormone is given subcutaneously. Distention of the uterine horn seems to restrain the effect of luteohormone. The mucous changes are not so marked if the hormone is deposited under the peritoneum around the horn, as contracted with the administration into the uterus or subcutaneously. This increased activity of luteohormone, when injected into

the uterus, leads us to assume that such a method of administration could be used as the one for the test of the hormone.

The induction of brooding behavior in the Jewel Fish. Noble, G. K., K. F. Kumpf and V. N. Billings. To appear in this Journal.

Brooding behavior in the cichlid, *Hemichromis bimaculatus,* with spawning experience can be produced experimentally by treatment with a number of substances. Most effective, in order, when a large series of fish is tested, are corpus luteum, proluton, and prolactin. Anterior pituitary extract, fresh fish pituitary, thyroxin, desiccated thyroid and phenol in 0.1 or 0.5% solution are effective to a much less extent. Sexually mature fish without spawning experience cannot be induced to brood with any of the above substances. Previous brooding experience is not essential, but greatly increases the response following treatment. At the beginning of their breeding cycle fish require less prolactin than those about to spawn; castrates require less than normals; and males have a lower threshold to prolactin than do females.

Hypophysis in protein metabolism. Is the pituitary factor active in protein metabolism identical with the growth hormone? Paschkis, K. E. To appear in this Journal.

The existence of a protein-metabolism hormone of the pituitary anterior lobe can be assumed: it lowers the blood amino-N and the blood urea-N level of rats, dogs and guinea pigs after administration of alkaline pituitary extracts. It prevents the rise of blood amino acids if, 3 hours after a first administration of 50 gm. of gelatine, a second administration of the same quantity follows. We call this phenomenon the 'regulation effect' in protein metabolism. In 3 cases of Simmonds's pituitary cachexia this regulation effect was missing. In 2 cases of pituitary nanosomia the regulation effect was normal. This is considered proof that the protein metabolism hormone is not identical with the growth hormone. Regulation effect was missing in one case of acromegaly previously published; it was normal in a new case of acromegaly.

Innervation of the hypophysis. Rasmussen, A. T. To appear in this Journal.

Relevant literature is reviewed and found to contain many disagreements. Comparatively little has been done directly on the human hypophysis. The author studied specific silver preparations of 48 human glands and of many laboratory animals. Nerves are as plentiful in the hypophysis of man as in that of animals. At least 50,000 fine, unmyelinated nerve fibers descend from the hypothalamus into the infundibulum, and there are that many cells in one supraoptic nucleus, which seems all out of proportion to the number of cells in the posterior lobe of the hypophysis. These fibers form an exceedingly dense plexus in the neural lobe, making it impossible morphologically to exclude abundant contact between the nerve fibers and the cells in their meshes. However, these cells have never been proven secretory, and a neurohumoral mechanism may be involved. Only an occasional nerve fiber from this source was seen among the epithelial cords of pars tuberalis and pars infundibularis. Large areas of pars intermedia appear to be devoid of nerve fibers, whereas other areas contain abundant fibers which, however, are not necessarily functionally related to the gland cells. Although many appearances in pars intermedia suggest nerve terminals, serial sections show that most of them are apparent only. A few terminals cannot be thus explained away. Only a negligible number of infundibular fibers get into the anterior lobe.

Small unmyelinated nerves are especially numerous in the capsule on upper surface of the anterior lobe. These are derived from the cavernous sympathetic plexus. The total number of fibers is estimated to be about 200. A few ascend along the vessels of the stalk. Many pass into the vascular stroma of the upper part of the anterior lobe. A prominent bundle just lateral to the stalk penetrates deeply into each half of the anterior lobe, more or less in association with blood vessels, but ultimately some of the fibers course among the secretory cells in limited regions. Small nerves pass downward in the capsule, and at numerous points a few fibers enter the superficial region of the anterior lobe and follow blood vessels into the posterior lobe. So much of the anterior lobe is apparently free from nerve fibers that secretory nerves to this part of the hypophysis remain highly questionable.

The treatment of a cretin with anterior pituitary extract. Rudolf, G. de M. To appear in this Journal.

A cretin who, following operation for mastoiditis, became apathetic on the administration of thyroid in 2 series, and also during enlargement of the thyroid gland accompanying frequent doses of antuitrin-G showed marked improvement, following the administration of

antuitrin-G. in small doses of 1 cc. and 2 cc. at weekly and fortnightly intervals, during each of 2 courses of treatment.

Unusual results following the injection of epinephrin. Rudolf, G. de M. To appear in this Journal.

Decreases of the pressor effect of epinephrin and increases of the depressor effect were recorded in 7 subjects following injections of the same substance given from 7 to 36 days previously.

On the reliability of present methods for characterizing two gonadotropic hormones, follicle-stimulator and luteinizer. Saunders, F. J., and H. H. Cole. To appear in this Journal.

A series of experiments was devised to determine any possible specific effects of pituitary luteinizer, LH. Using pituitary synergist, the *in vitro* combination with LH did not produce any greater augmentation than similar combination with egg albumin or zinc sulfate. With all of these, augmentation was obtained only when the combination with the synergist was made *in vitro,* not when the two were injected separately or successively. Some pituitary follicle-stimulating extracts, FSH, when administered to 21-day-old female rats in 8 injections, produced only ripe follicles at necropsy 5 days after the first injection. However, the same total dose, administered in 3 injections, produced corpora. The addition of LH inhibited estrous smears in these rats, but not in rats simultaneously injected with estrin. Mare gonadotropic hormone produces only follicular development when injected into 18-day-old rats. Addition of pituitary LH to this serum, or injection of LH following an injection of serum in rats of this age, failed to produce corpora. One can obtain from the pituitary 2 gonadotropic extracts, each having somewhat different effects. Since, however, augmentation is also produced by egg albumin and zinc sulfate, and since the production or non-production of corpora is dependent upon the manner of injection and the type of the test animal as well as the type of the extract, it seems necessary to devise more specific means of characterizing the LH fraction before it can be considered a separate hormone.

Hormone studies with the ultra-centrifuge. II. The concentration of anterior lobe and pituitary-like hormones with the ultra-centrifuge. Severinghaus, Aura E., L. Levin and J. A. Chiles, Jr. To appear in this Journal.

The gonadotropic hormones of pregnant mare serum (Antex), of castrate urine, and a thyrotropic flavianate extract of beef hypophysis have been subjected to 150,000-200,000 g. in an ultra-centrifuge of improved design for periods of 4 to 6 hours. A marked concentration of the mare serum and castrate urine preparations was affected, but little if any concentration of the thyrotropic flavianate extract was obtained. In all instances a sludge was thrown down in the base of the tubes in the form of a gelatinous pellet. These pellets were found to be without gonadotropic or thyrotropic potency. Their elimination represented a mechanical purification of the preparations. The centrifuged gonadotropic extracts were assayed by injection into 21-day-old female mice, the weights of ovaries and uteri being the measure of gonad-stimulating potency. The thyrotropic potency was assayed by the weight response of thyroids of injected, day-old chicks. It was demonstrated that braking the centrifuge to accomplish a comparatively rapid deceleration, after the desired run, interfered with the concentrations of the extracts. The experiments demonstrate the feasibility of concentration and purification of hormone preparations by physical methods, and suggest the possibility of actual hormone separations by the same means. Such experiments are now in progress.

The effect of testosterone-propionate on the X-zone of the mouse adrenal. Starkey, W. F., and E. C. H. Schmidt, Jr. To appear in this Journal.

A study has been made of the adrenal glands of 247 Albino mice (141 experimental and 106 control animals) of a standard strain maintained under standard conditions. Variations in adrenal conditions were found to be fewer in mice of a standardized strain, but were still found in sufficient numbers to require litter-mate controls for experimental comparison. Testosterone-propionate treatment over short periods of time has been found to cause X-zone disappearance in normal adolescent males and females, adult females, castrate adult males and adolescent and adult ovariectomized females. X-zone formation has been prevented in young male and female mice. The time required to cause experimental disappearance of the X-zone has been found to correspond to the periods required for normal disappearance.

Oral therapy in adrenal insufficiency. The efficacy of a concentrated adrenal cortical extract preserved in glycerol. Thorn, G. W., K. Emerson, Jr. and H. Eisenberg. To appear in this Journal.

The oral administration of a concentrated extract of the adrenal cortex preserved in glycerol has resulted in a decrease in the renal excretion of sodium and chloride and an increased potassium excretion in patients with Addison's disease and in bilaterally adrenalectomized dogs. Glycerol-extract treatment has prevented the usual sodium and chloride diuresis which follows bilateral adrenalectomy in the dog. Bilaterally adrenalectomized dogs have been maintained successfully on the glycerol preparation for prolonged periods. In man and dog the ratio of extract administered twice daily by mouth, as compared to the extract administered twice daily by injection, is approximately 2.5:1. No toxic effects have been observed from the administration of 10 to 20 cc. of glycerol per day. Extract, administered orally, should not be used in the treatment of acute Addisonian crisis.

ADRENALS

The rat in the assay of cortin. D'Amour, F. E. and Dorothy Funk, J. Pharmacol. & Exper. Therap. **62**:307. 1938.

In experiments, using 422 male adrenalectomized 30-day-old rats not treated with cortin, neither the length of survival nor the weight was influenced by a variety of procedures, except when the salt intake was increased. Cortin, evidently, is either rapidly destroyed or eliminated because extract from 500 cc. steer blood per day was ineffective in prolongation of life. Various experimental and commercial extracts were assayed, using 30-day-old male adrenalectomized rats. The results indicate the desirability of a standardized assay technic. The oral administration of cortin is as effective as parenteral. The rat unit suggested in the amount of material per day per rat necessary to produce a net gain in weight of 10% over a period of 10 days.—E. L.

Factors affecting the uptake of water by frogs when injected with extract of the posterior hypophysis. Boyd, E. M., and G. M. Brown, Am. J. Physiol. **122**:191. 1938.

The effect of posterior hypophysis extract on the body weight of the frog was determined under a variety of conditions. From 6-36 frogs were used to determine the effect of each variation of each factor with additional 1/3-2/3, this number serving as controls. All results were statistically treated. The site of injection, body weight, difference in species within the genus Rana, sex, and the volume of water in the frog bath had little or no effect on the percentage of increase in weight of the frogs following posterior lobe extract. In a dark room the gain in weight with extract injection after 3 hr. was about 27% as compared with an increase of 10% in the sunlight. At lower temperature the increase in weight was somewhat larger, and the effect lasted a considerably longer time. Salts (NaCl, Na_2HPO_4, NaH_2PO_4 and KCl) in concentration greater than 0.4% inhibited the reaction; pH 7.0 was found to be optimum for the reaction. Various commercial extracts in similar doses produced relatively similar results. Pitocin produced much more effect than Pitressin.—L. A. Lewis.

Carbohydrate and electrolyte changes in adrenal insufficiency in the dog. Britton, S. W., H. Silvette and R. Kline, Am. J. Physiol. **122**:446. 1938.

Male and female dogs were used in these experiments. After adrenalectomy the survival period of the untreated dog was as long as 21 days. The blood sugar was reduced to 59 mg.% as compared with 89 mg.% in the normal dog. The liver glycogen of the dog in insufficiency was decreased to 0.20% (average) as compared with the normal of 1.50%; the muscle glycogen to 0.41% as compared with 0.77%, and the heart muscle glycogen to 0.26% as compared to 0.73%. The serum electrolyte changes corresponded with those reported elsewhere in the literature for adrenalectomizd dogs. The blood sugar was increased following hormone therapy. Adrenalectomized dogs treated with physiologically normal saline had a normal serum Na and serum Cl, but the sugar level fell, and insufficiency developed. Adrenalin had no consistent effect on the blood sugar of dogs in the presymptom stage. A table summarizing the outstanding findings in adrenal insufficiency was given.—Lena Lewis.

Hypertrophy and hyperplasia of the adrenals (Hypertrophie et hyperplasie des capsules surrénales). Caussade, M. G., Ann. d'anat. path. **14**:649. 1937.

Injection of adrenal-cortical extract in 2 guinea-pigs appeared to cause some hypertrophy and hyperplasia of the glomerular zone of the adrenal cortex.—A. T. R.

Effect of administration of adrenal cortical hormone preparation on fertility, pregnancy and lactation in the normal rat. Hall, V. E., P. E. Chamberlin and O. H. Müller, Am. J. Physiol. **122**:16. 1938.

The effects of adrenal cortical hormone preparations on the incidence of pregnancy and/or litter weights of rats treated before and during pregnancy and during lactation were noted. Although in general the effects of the hormone seemed to be one of increasing reproductive activity, in most cases the differences over controls were not statistically reliable. Clearest results were obtained for pregnant animals maintained on a low sodium-high potassium diet, in which case the hormone preparations tended to restore litter weights to a normal value from the low values caused by the diet. It is suggested that this effect is due to the activity of cortical extracts in causing increased potassium and decreased sodium excretion in normal animals. The data also showed that the hormone preparations tended to increase the incidence of pregnancy and the number of fetuses in animals on a normal diet. The duration of gestation and litter weight were not affected in these animals. No significant effects on the lactation capacity of the rats either on normal or low sodium-high potassium diets were produced by the hormone injections.—S. Cohen.

The effects of adrenalectomy upon the water exchange of cats with diabetes insipidus. Ingram, W. R., and C. A. Winter, Am. J. Physiol. **122**:143. 1938.

Diabetes insipidus was produced in cats by producing lesions in the hypothalamus which resulted in atrophy of the supraoptico-hypophyseal tract and atrophy of the pars nervosa of the pituitary. Seven such cats showing diabetes insipidus were adrenalectomized. Four control adrenalectomized cats were also studied. Water exchange before and after adrenalectomy was studied in each case, and in some cases the water exchange was also determined when the cats were maintained on cortin. The H_2O exchange of 3 cats showing diabetes insipidus was studied while on a restricted water intake. Following adrenalectomy in the diabetic insipidus animal a decrease in H_2O intake and a resulting negative water balance was observed. Cortin maintained the water exchange at a normal level. The negative water balance was no greater than in ordinary adrenalectomized cats and is not in itself sufficient to produce death. Diabetic insipidus cats can suffer greater loss of water without fatality than was fatal to the adrenalectomized diabetic insipidus cats. The survival time of adrenalectomized animals with diabetes insipidus was shorter than that of the simple adrenalectomized cat.—L. A. Lewis.

Influence of adrenalectomy upon ketolytic activity. MacKay, E. M., and R. H. Barnes, Am. J. Physiol. **122**:101. 1938.

The purpose of these experiments was to determine the effect of adrenalectomy on the oxidation of exogenous ketones. To this end rats, 180-290 gm. in weight, were studied in pairs, 1 adrenalectomized and 1 control, 22 such pairs of males and 14 pairs of females being used. The procedure was to give a measured quantity of sodium aceto-acetate or sodium β-hydroxybutyrate by stomach tube to fasting rats in 2 daily doses and follow the keytone excretion. The period was usually 4 days. From 50-200 mg. of the keto-esters were administered daily in different experiments. Total urinary ketone bodies were determined by Van Slyke's method. The ketonuria produced by feeding the sodium salts of diacetic or β-hydroxybutyric acid to fasting rats was significantly reduced by adrenalectomy, an observation leading to the conclusion that this procedure increased the ketolytic activity of the organism.—K. Brownell.

Sensitization of the adrenal gland by partial denervation. Simeone, F. A., Am. J. Physiol. **122**:186. 1938.

Partial denervation of the cat's adrenal increases the response of the adrenal medulla to nervous stimulation.—F. A. H.

ENDOCRINE-GENERAL

On the specificity and related properties of the crustacean chromatophorotropic hormone. Abramowitz, A. A., and R. K. Abramowitz, Biol. Bull. **74**:278. 1938.

From assays on the blinded Uca the authors estimated that 0.2 γ of hormone is present in each eye-stalk. Although distilled water produced melanophore expansion due to its hypotonicity, a series of 16 drugs had no such effects. Of the organic solvents investigated for extracting the hormone only 95% ethyl alcohol, 95% methyl alcohol and absolute methyl alcohol extracted more than 50% of the activity.—E. L.

Effects of amniotin or theelin on metabolism of liver and kidney. Andersen, Dorothy H., and J. Victor, Am. J. Physiol. **122**:113. 1938.

Sixty to 62-day-old rats were ovariectomized. Six weeks after operation amniotin (Squibb) was injected in total dose of 30 R.U. over 1 or 2 days. In other cases crystalline theelin was injected in 30 R.U. doses. At various subsequent intervals the rats were sacrificed, and the metabolism of liver and kidney slices was determined in Ringer solution with a differential volumeter. Only in the liver were there metabolic changes. On the 3rd day after injection a significant rise in O_2 consumption was observed. Control Q_{O_2} values were 13.3 $\pm$ 0.40 with R.Q. of 0.774 $\pm$ 0.012. On the 3rd day after injection an average of 12 rats gave Q_{O_2} of 15.8 $\pm$ 0.14 with R.Q. of 0.771 $\pm$ 0.01. On the 4th day the respiration returned to control levels. The data, although having a large scatter, suggest a rise on the 2nd day after injection. Theelin directly increases respiration of anterior pituitary but not the liver of spayed rats. The 'in vivo' reaction is also different. The stimulation of pituitary metabolism reaches a peak 6 hr. after theelin injection, and returns to control value in 12 hr. A difference in stimulus mechanisms for the 2 tissues is suggested.—S. R. Tipton.

The rôles of anterior pituitary and thyroid in the stimulation of tissue metabolism following theelin administration. Victor, J., and Dorothy H. Andersen, Am. J. Physiol. **122**:167. 1938.

Spayed-thyroidectomized rats and hypophysectomized rats were injected with 30 R.U. of amniotin (Squibb) or theelin, subsequently sacrificed and metabolic measurements made on liver and kidney slices. No changes in metabolism were observed even 4 days after hormone, suggesting that both hypophysis and thyroid are essential for the metabolic effect of theelin on liver tissue. Intraperitoneal injection of 30 γ of l-thyroxin into spayed rats resulted in significant increase in liver metabolism 2 days after injection of hormone. Intraperitoneal injection of 100 mg. thyrotropic hormone into spayed rats resulted in significant increase in liver and kidney metabolism on the 3rd day after injection of hormone. Thyrotropic hormone preparation contained gonadotropic, adrenotropic and growth promoting factors. The lag periods, following thyroxin, anterior pituitary extract and theelin, are 2, 3 and 3 days respectively. Intervals indicate that theelin stimulates pituitary, and anterior lobe secretion stimulates the thyroid, which in turn stimulates the liver metabolism.—S. R. Tipton.

GONADS

Thyroid and metropathia hemorrhagica (Tiroides y metropatía hemorrágica). Araya, R., Semana méd. **45**:636. 1938.

The author asserts on the basis of histological studies of operative material that ovulation may occur at any time of the sex cycle and that ripe follicles and corpora lutea may also be found coexistent with any stage of the uterine mucosa. Metrorrhagias are treated with follicular and thyroid hormones combined. A high percentage of 'cures' is claimed, but in 50% the bleeding recurs later on.—A. E. M.

The effects of castration, theelin, testosterone and antuitrin-S on the lipoids of blood, liver and muscle of guinea pigs. Bogdanovitch, Sinisha B., and Evelyn B. Man, Am. J. Physiol. **122**:73. 1938.

A study of the relation of sex hormones on lipids of the whole blood, liver and muscle was made on male and female guinea pigs at various ages. The animals were divided into three age-groups: immature (I), 6-9 months, (II), and 13-25 months, (III). Normal female pigs of group II and III had higher blood cholesterol values than males of the same groups. Animals of group III as compared with group II exhibited higher blood and liver fatty acid values, as well as, higher liver phospholipid values. Theelin caused most marked increase in liver and blood fatty acids of female pigs. It was generally observed that injections of antuitrin-S resulted in elevation of blood liver and muscle fatty acids in both male and females.—K. P. McConnell.

The magnesium content of serum after prolan injections (Der Magnesiumgehalt im Serum nach Prolaninjektion). Borgström, S., Skandinav. Arch. f. Physiol. **78**:73. 1938.

Contrary to previous reports, the injection of 10 doses of 100 R.U. of prolan failed to affect the magnesium content of the blood serum of rabbits.—A. G.

A study of the mechanism whereby coitus excites the ovulation-producing activity of the rabbit's pituitary. Brooks, C. M., Am. J. Physiol. **121**:157. 1938.

Removal of the sympathetic chains failed to inhibit ovulation in response to subsequent

mating in 13 rabbits, demonstrating the non-essentiality of a sympathetic nerve supply to the hypophysis in relation to gonatotropic function. Lactation, moreover, occurred in such animals when allowed to litter. When, however, the pituitary stalk was severed in 16 animals, some of which retained intact sympathetic nervous systems, all of these failed to ovulate despite regular and frequent matings. Histological examination by the silver impregnation technic revealed scattered fibers, resembling nerve fibers, in the anterior lobes of normal rabbits; they appeared to enter the gland from the stalk. These fibers persisted after sympathectomy, but were never found after complete stalk transection. The pituitaries of the animals with transected pituitary stalks showed certain histological and circulatory abnormalities. No obvious failure of anterior pituitary function in these animals however, could be detected; i.e., there was little or no effect on the growth, the ovaries, thyroids, adrenals and genital tracts, and on the carbohydrate metabolism of animals in which the pituitary stalks were severed. Seven rabbits, in which the stalks were only partially transected, ovulated only after many matings within a brief period of time; the pituitary of some of these animals showed the circulatory and histological changes noted after complete stalk transection, but a few scattered nerve fibers were noted. The author believes these experiments support the hypothesis that in the normal rabbit a nervous stimulation of the pituitary occurs on mating, and that the nerves involved pass down the stalk from the hypothalamus to the anterior lobe.—S. L. Cohen.

Action of male hormone on the glands of the prepuce (Action de l'hormone male sur les glandes préputiales). Buillard, H., and A. Ravina, Compt. rend. Soc. de Biol. **125**:965. 1937.

Immature male rats (20 to 48 gm.) were injected subcutaneously every other day with 1.6 to 2.5 mg. of testosterone propionate. In 2 weeks the glands of the prepuce, which are paired organs and enlarge greatly at the time of puberty, had developed until they were 6 times the weight of those of the controls. With the increase in size went a precocious histological and physiological maturity. Cowper's glands, likewise, were stimulated to premature development by this treatment.—J. C. D.

Endocrine influence on the human fallopian tube mucosa and its therapeutic employment (Ueber die hormonale Beeinflussung der menschlichen Tubenschleimhaut und ihre therapeutische Ausnutzung). Caffier, P., Zentralbl. f. Gynäk. **62**:1024. 1938.

Women who were to have extirpation of the tubes were given preliminary treatment with 5 daily doses of 50,000 U of estrin benzoate or with 2 Rb.U. of progestin and the tubes and curettage material taken on the 6th or 7th days. It was evident that the tubal mucosa participates in the changes of the types well known for the endometrium. Suggestions are made for the conservative treatment of tubal pregnancy by using this knowledge. The use of such endocrine therapy also assisted in securing viable transplants of tubes.—E. L. S.

The bioassay of mare serum hormone. A comparison of ovarian and uterine weight methods. Cartland, G. F., and F. W. Nelson, Am. J. Physiol. **122**:201. 1938.

Assay curves for mare serum hormone were constructed by plotting ovarian and uterine weights at increasing dosages of the hormone administered to large groups of immature rats of 21-23 days of age. A purified hormone preparation, free of estrogenic substances, was employed. Maximum effect occurred 96 hr. after the first of 3 daily injections. Maximum mean ovarian weights more than 15 times that of the controls, and uterine weights 8 times that of the controls were observed. The uterine weight increases are attributed to the stimulation caused by estrogenic hormones produced in the ovaries as a result of follicle stimulation by the mare serum hormone. Although much more sensitive to small amounts of hormone than ovarian weight, the uterine weight response can be used on only a very small dosage range. On the other hand, with sufficient stimulation the ovarian response is practically a straight line function of dosage. Since this is also a region of high sensitivity to small changes in dosage, the ovarian curve is most suitable for the quantitative assay of the hormone. An ovarian and uterine rat unit are defined. Luteinization in the ovaries was found to be a regular function of dosage of the hormone. It was also observed that the injections of male serum hormone caused no significant effect on the kidneys, adrenals, thyroid and hypophysis.—S. Cohen.

Production of ovarian tumors by folliculin (Production de tumeurs ovariennes par la folliculin). Champy, C., Compt. rend. Soc. de Biol. **125**:634. 1937.

In guinea pigs, rats and rabbits, prolonged injection of folliculin or testicular lipoids brought about the formation of cysts in the ovary. These resembled the mucoid cysts found in human ovaries.—J. C. D.

Artificially stimulated tubal growth, a participating factor in relief of sterility with estrin (Künstlich erzeugtes Tubenwachstum, ein mitbedingender Faktor bei der Behebung von Sterilität durch Follikelhormon). Clauberg, C., Zentralbl. f. Gynäk. **62**:1034. 1938.

Estrin treatment of rabbits and of women leads to growth of the fallopian tubes as well as the uterus. This was found to increase the mucosal development of a chronically inflamed tube. The effects mentioned may be useful in the treatment of sterility—E. L. S.

Studies on the functional relations between the ovarian hormone in primates (Etude des rapports fonctionnels entre les hormones ovariennes chez les Primates). Currier, R., and G. Gros, Compt. rend. Soc. de Biol. **125**:746. 1937.

Algerian monkeys were spayed when 2 years old, which is 3 years before normal puberty. Injections of folliculin brought on congestion of the sexual skin. If this folliculin was followed by progestin alone, this congestion disappeared rapidly. Addition of folliculin to the progestin reduced its effectiveness, but, in a proportion of 1 part of folliculin to 6 parts of progestin, it was still active. When tested on rabbits, 1 part of folliculin neutralizes the action of 40 parts of progestion, whereas in rats the proportion is 1 to 125. The antagonism between these two is, therefore, not a simple chemical reaction but depends on factors which vary with the test animal used.—J. C. D.

The assay of gonad-stimulating preparations. D'Amour, Marie C., and F. E. D'Amour, J. Pharmacol. & Exper. Therap. **62**:263. 1938.

The ovaries or seminal vesicles of 21-day-old rats, which had received 3 daily doses of a stock pregnancy urine preparation, were weighed. Two mg. of this preparation produced an almost maximal response of the seminal vesicles, 3 x the weight of the untreated controls, whereas in females 24 mg. was necessary to produce the same rate of increase in ovarian response. Placental extracts contained only small quantities of gonad-stimulating substances. Pituitary preparations could not be assayed satisfactorily on males, as excessively large doses were required, but the ovarian response was higher than that obtained with urine preparations. The corpus luteum and opening of the vagina methods were not satisfactory as assay procedures. Although the authors also consider the estrous smear method satisfactory for urine, they believe, from their experience with 1600 animals, that the ovarian weight method of assay is preferable. Of the 10 commercial preparations assayed using the increased weight of the seminal vesicles as a test, only 2 urinary and 1 placental preparation were assayed satisfactorily.—E. L.

The effects of various gonadotropic substances upon the ovaries, pituitaries and adrenals of animals receiving long-term injections of estrin. Diaz, J. T., Doris Phelps, E. T. Ellison and J. C. Burch, Am. J. Physiol. **121**:794. 1938.

Mature female rats were injected daily with estradiol benzoate for 60-70 days; some of the animals then received simultaneous injections of gonadotropic hormone for 5-10 days, and the ovaries, pituitaries and adrenals of these animals finally removed and examined. The estrous cycles of some of these long-term treated animals were also examined. The ovaries of the estrin-treated animals showed a marked decrease in size with a general regression of germinal and interstitial elements. These atrophic changes were all absent in those animals simultaneously treated with gonadotropic substance. No qualitative differences in the effects of various gonadotropic preparations could be detected. The gonadotropic hormone treatment also changed the vaginal smears from the cornified cell type to one in which a mixture of leucocytes and mediated epithelial cells predominated. The estrin injections caused marked pituitary hypertrophy, but no significant change in the adrenals. The addition of gonadotropic hormone usually caused further hypertrophy of the pituitary and slight hypertrophy of the adrenals. The authors believe this evidence indicates that the estrin acts directly on the pituitary, causing, after long periods of injection, an exhaustion of the gonadotropic hormone production by the gland. Atrophy, but not damage, of the ovary then occurs owing to inadequate stimulation.—S. Cohen.

The treatment of habitual abortion by progesterone. Elden, C. A., Am. J. Obst. & Gyn. **35**:648. 1938.

Eight women with a history of 1 or more spontaneous abortions were treated with from 10 to 44 I.U. progesterone during the first six lunar months. No other endocrine therapy was

used. Six of the 8 delivered normal children. One failure was unaccounted for; the other was due to an operation for laceration of the cervix. Smaller amounts of progesterone may be effective in carrying a pregnancy to term.—Author's Abstract.

The use of progestin in obstetrical complications. Falls, F. H., South. M. J. 31:556. 1938.

In 60 cases in which over-excitability of the uterus seemed to be the controlling factor, progestin was given to control the contractions. Five failed to respond, and 5 aborted. In the latter, the fetus was evidently dead before treatment was begun. The remaining 50 responded well, and were able to carry their fetuses until they were of viable age. In the same way, 5 of 8 cases of partial premature separation of the placenta were carried through the critical period. Several other conditions are suggested in which the inhibitory action of progestin may be of clinical value. The authors used one rabbit unit twice a day for active treatment. If injected deep into the muscle of the thigh, there was little local reaction. No general ill effects from its use were seen. It did not interfere with uterine contractions in normal delivery or in the expulsion of a dead fetus. Preparations lost their potency rapidly when stored.—J. C. D.

The differential action of pituitary gonadotropic hormones upon the secretory capacity of the graafian follicle and corpus luteum. Foster, M. A., Am. J. Physiol. 121:633. 1938.

The differential effects of administration of follicle stimulating (FSH) and luteinizing (LH) pituitary (sheep) extracts upon the motility and pituitrin sensitivity of the uteri of 108 juvenile rabbits, 12-14 weeks old, was studied. The gonadotropic preparations were injected subcutaneously over a period of 3-5 days, when uterine biopsies were made for the motility and sensitivity tests. It was found that the administration of FSH to juvenile rabbits caused spontaneous motility and marked pituitrin sensitivity of uteri normally non-motile. This was interpreted as due to estrin produced in the graafian follicles as a result of the FSH stimulation. LH treatment resulted in luteinization, with the uterus developing a motile state characteristic of pseudopregnancy. FSH plus a trace of LH resulted in spontaneous ovulation, but larger doses of LH were necessary before inhibition to pituitrin developed; further increase in LH resulted in pituitrin inhibition and also, in most cases, loss of spontaneous motility. The artificial pseudopregnancy following experimental ovulation was found to last 13-14 days in juvenile rabbits; injection of FSH shortened this period to 6 days or less, and LH injections limited it to 9 days. These effects were ascribed to estrin secretion and inhibition of luteinization following the FSH treatment, and to overstimulation and injury to the lutein tissue by the LH extract. Mated adult females injected with FSH or LH in the same dosages failed to implant; progestational changes lasted 2-13 days, and motility reactions were found to be similar to those found in the immature animals.—S. L. Cohen.

The interrelationship of the pituitary gonadotropic hormones in follicular development and ovulation of the juvenile rabbit. Foster, M. A., and H. L. Fevold, Am. J. Physiol. 121:625. 1938.

One hundred and fifty immature, 12-14 week old rabbits were used to study the artificial follicular development induced by subcutaneous injection of follicle-stimulating pituitary extracts (FSH) alone and combined with increasing amounts of luteinizing pituitary extracts (LH). The gonadotropic preparations used were prepared from horse, sheep and hog pituitary powder by methods previously described. FSH was the only preparation capable of inducing the development of normal follicles with no other effects. Where traces of LH were injected subcutaneously with the FSH, most of the animals ovulated spontaneously. When higher concentrations of LH were present, atresia, cystic degeneration and luteinization occurred; experimental ovulation under these conditions always failed. Following adequate follicular development by subcutaneous injections of FSH, the intravenous injection of FSH alone frequently, but not always, induced ovulation, whereas FSH plus traces of LH always caused ovulation. The use of horse preparations resulted in an average of 38, sheep in 22, and hog in only 7 ovulation points. Increasing the LH in the intravenous mixture (FSH dosage constant) caused a decrease in the number of ovulation points; when sufficient LH was present, ovulation failed together. Extracts of pregnant mare's serum injected subcutaneously were found to stimulate only a small number of follicles; intravenous injection resulted in ovulation of only 2-4 follicles without increasing ovarian size. Pregnancy urine preparations were unsatisfactory for follicular development, but could be satisfactorily substituted for LH in the intravenous mixture.—S. L. Cohen.

The immature rat uterus as an assay end-point for gonadotropic substances. Heller, C. G., H. Lauson and E. L. Sevringhaus, Am. J. Physiol. **121**:364. 1938.

In the assay of aqueous suspensions made from pooled pituitary glands of 57 mature female rats, castrated 20 days previously, 228 immature female rats, 21 days old, were employed. Fifteen animals on each of 14 closely-graded dose levels were injected twice daily for 4½ days. The division of the dose into 2 daily injections was found to give a more sensitive and uniform test than a single daily injection. The degree of uterine enlargement above control size was judged by gross inspection. Vaginal, uterine and ovarian weights were recorded and vaginal opening observed. Units are defined for the effect of the gonadotropic preparation on each of these organs, and curves constructed showing the relationship of dose to ovarian uterine and vaginal weights. The minimal doses of the gonadotropic preparation producing uterine enlargement and uterine and vaginal weight increase were found to be about ⅓ to ½ as large as the minimal dose causing vaginal opening, and only ⅛ as large as the minimal dose which resulted in ovary weight increase. On the basis of sensitivity, dependability and ease of measurement, the uterine weight was considered the most satisfactory gonadotropic end-point. Histological examination of the vagina, in order to determine epithelial thickness, substantiated the uterine weight changes. Some evidence was obtained indicating a variation in the ratio between uterine and ovarian units and in the shape of the uterine weight curve for different gonadotropic preparations, depending upon the source.—S. L. Cohen.

The effect of male hormones on the seminal vesicles of the rat. II. Glandular epithelium (Die Wirkung der männlichen hormone auf die Vesiculardruse der Ratte. II. Drüsenepithel). Jacobsen, E., Skandinav. Arch. f. Physiol. **78**:170. 1938.

The response of the epithelium of the seminal vesicle of rats to the injection of male hormones reflects the changes occurring in the weight of the whole organ. However, the final effects on the epithelium are attained much sooner than are the weight changes.—A. G.

The effect of male hormones on the seminal vesicles of the rat. I. (Die Wirkung der männlichen Hormone auf die Vesiculardrüse der Ratte). Jacobsen, E., and J. T. Christensen, Skandinav. Arch. f. Physiol. **78**:155. 1938.

The response of the seminal vesicular growth to the injection of androsterone, androstendiol, testosterone and testosterone propionate was studied in 600 castrated rats. The growth response of the seminal vesicles (as determined by their weight) varied with the size and age of the rats. The relative increase in size with varying doses gradually decreased with increased dosage.—A. G.

Influence on the duration of gestation of the injection of pregnancy urine extract in the rat before and after implantation. King, Jessie L., Am. J. Physiol. **122**:455. 1938.

The effect of a single intraperitoneal injection of 50 R.U. (0.5 cc.) of antuitrin-S on the course of pregnancy and on parturition in pregnant rats was observed. There was little or no effect in a group of 17 animals in which the injection was made prior to the 6th day of pregnancy (pre-implantation). Nearly all of the group of 24 rats injected between the 7th and 11th days of pregnancy (post-implantation) showed a prolongation of gestation of 2 to 3 days. All of this group delivered abnormal fetuses: some showed a postmature condition; many were dead. The extract causes the luteinization of many of the follicles in the ovaries, and the animals which had undergone delayed parturition showed many more luteinized follicles than there were fetuses. In only 3 cases did rats, which had undergone the delayed parturition, show a permanent impairment of the uterus. These results are in conformity with those obtained by other workers, and indicate that parturition in the rat is under hormonal control.—S. Cohen.

General muscular hypertrophy induced by androgenic hormone. Papanicolaou, G. N., and E. A. Falk, Science **87**:238. 1938.

In the adult guinea pig the temporal muscles in females are comparatively small and flat but in males form distinct protuberances. It was found that treatment of the female subjects with gonadotropic hormone (follutein) resulted in considerable hypertrophy of the temporal muscles so that after several weeks they were as large as those of males. Other muscles of the body were similarly affected. The treatment was ineffective in castrated males and spayed females, hence the primary gonads appear to be necessary to the reaction. The ovaries of treated animals showed considerable hyperplasia of the interstitial tissue. In both males and females deprived of primary gonads testosterone propionate caused muscular hypertrophy. Progesterone and estrogen (amniotin) gave negative results, but when androgenic hormone was combined

with either general muscular hypertrophy resulted. These findings suggest that the higher muscular development of males is due to their androgenic hormones. The suggestion that these hormones may be of therapeutic use in muscular deficiencies is obvious.—R. G. H.

Effects of folliculin during pregnancy (La administración de foliculina en el embarazo. Estudio experimental). Peralta R. A., and E. Colombo, Bol. Acad. nacdi. med. Buenos Aires P. 292, 1937.

Large doses (10-20,000 U) of dihidrofolliculin benzoate were injected into rabbits during the first 12 days of pregnancy, while the ovular sacs were detached but retained in an immobile uterus. Necrobiosis subsequently occurred. Smaller doses (100 U) produced death and reabsorption of some of the embryos. During the second half of pregnancy (14-19 days) folliculin produced abortion, and single doses of 100 U evacuated the uterus in 6-7 days. Repetition or increase of the dose did not accelerate the abortion. The effect is attributed to a disturbance in the folliculin-placental progesterone ratio, and not to neutralization of the progesterone produced by the corpus luteum.—J. T. L.

The value of correlating hormonal tests with histologic sections in tumor of the testis. Powell, T. O., J. Urol. 39:522. 1938.

Embryonal tumors of the testis may be simply and conveniently grouped to represent grades to the clinician on the basis of the probable outcome to the patient who receives average treatment. In order to grade the embryonal tumors it is necessary to make *a)*, hormonal tests upon the patient's urine before sugery or irradiation; *b)*, fresh non-irradiated tumor tissue for hormonal extraction and histologic study; *c)*, hormonal tests following surgery and irradiation. The successful grading of the embryonal tumors depends upon correlating the histologic pattern of the tumor with the total hormonal information. The determination of the grades assigned to the groups here proposed is based upon the clinical study of 74 cases of tumors of the testis, followed from 2 to 4 years. Certain data of interest, deduced by the author from the study of this material, are listed briefly without proof of discussion.—Author's Summary.

The detection and measurement of the electrical concomitant of human ovulation by use of the vacuum-tube potentiometer. Rock, J., J. Reboul and H. C. Wiggers, New England J. Med. 217:654. 1937.

By means of electrodes placed on the abdomen and in the vagina the electrical potential was measured with a vacuum tube potentiometer. For the 40 hours preceding ovulation the potentials recorded were about 2 millivolts. At the time of ovulation the voltage increased sharply to 7 to 8 millivolts. The voltage remained elevated about 8 hours, reaching a maximum about the 4th or 5th hour. It then fell to the pre-ovulation level and remained nearly constant for the next 10 hours. Laparotomy after 14 hours showed a freshly ruptured follicle which was excised and examined histologically. Evidences of luteinization were already present in the follicles.—J. M. L.

Noteworthy forms of menstrual disturbances and their influence by hormones (Bemerkungswerte Formen von Ausfallserscheinungen und ihre hormonale Beeinflussung). Rust, W., Zentralbl. f. Gynäk. 62:165. 1938.

These are 3 cases of amenorrhea treated with estrin. One was of pruritis and other climacteric symptoms, with 12 years amenorrhea, restored to comfort and cyclic bleeding. Another, one of the involutional type following hysterectomy with ovaries left intact, was completely relieved. The third was one of amenorrhea and sterility at the age of 35, with some evidences of virilism; after use of cycles of estrin therapy, 1.0 mg. every third day for 21 days of each of 3 months, she became pregnant and went to term. The pregnancy began within the next 6 months.—E. L. S.

Unilateral oophorectomy and the climacteric (Ueber einseitige Eierstockentfernung und Klimakterium). Schmid-Burgk, L., Zentrlbl. f. Gynak. 62:982. 1938.

From a series of 519 cases follow-up data were secured from 174. Of these 113 had no dysmenorrhea or climacteric complaints either before or after the operation. Of this group 75% had no change in menstrual characteristics. In the 61 cases with complaints, the difficulties tended to be reduced when menses were made more regular, and to be increased when menses became less regular after the operation. There were postoperative breast complaints in 22 cases, premature climacteric obesity in 22, and vasomotor complaints in 10. A total of 21 appeared to enter the climacteric earlier than the average (47).—E. L. S.

Disturbance of fat metabolism under the influence of estrone: a tumorous fat accumulation in the parametrium experimentally produced by treatment with estrone on castrated guinea-pigs. Störtebecker, T. P., Skindinav. Arch. f. Physiol. **78**:268. 1938.

A tumerous fat accumulation (consisting of adipose tissue, connective tissue and phagocytic cells) forms in the parametrium of guinea-pigs injected with estrone following castration or atrophy of the ovaries. Fatty infiltration of the liver occurs under the same conditions.—A. G.

Treatment of endoangiitis obliterans and ulcers with sex hormones (El tratamiento con hormonas. El tratamiento de la endoangeitis obliterante y de las úlceras tórpidas de las piernas con hormona sexual). Teitge, H., Semana méd. **45**:603. 1938.

Endoangiitis obliterans and leg ulcers are favorably influenced by estrin. Circulatory disturbances manifesting themselves by cold and numbness in the lims also respond satisfactorily, whereas diabetic gangrene and Raynaud's disease are not improved. The male sex hormones have a similar action; both hormones are effective in both sexes showing that the action is not specific.—A. E. M.

Treatment of gonococcic vaginitis with estrogenic hormone: further studies. Te Linde, R. W., J.A.M.A. **110**:14. 1938.

The author treated 175 children with gonococcic vaginitis by amniotin in suppository form. No patient failed to recover. The average time for epithelial response was approximately 2 weeks. Rarely as long as a month was necessary before the action of the estrogen could be noted, whereas occasionally a full response was seen in 1 week. The suppositories contained 1,000 I.U. One suppository was introduced daily at bedtime. In the average case, the entire treatment extended over about a month. Of the first 100 cured patients 98 were well from 3 months to 2½ years after cessation of treatment. All of the children seem to be developing normally; no evidence of harm was seen. The author feels that the increased acidity brought about in the vagina by the action of the estrogen is a factor in overcoming the infection, but since his results were not nearly so good when acidifying suppositories of floraquin (each suppository contained 1½ gr. (0.1 gm.) of diodoquin, lactose, dextrose and boric acid) were employed, he believes that amniotin introduces an additional factor probably the covering of the vagina with thick epithelium.—R. G. H.

Histologic demonstration of the activity of percutaneously administered follicle hormone in kraurosis vulvae (Ueber den histologischen Nachweis der Wirkung perkutan zugeführten Follikelhormons bei der Kraurosis vulvae). Tscherne, E., Zentralbl. f. Gynäk. **62**:169. 1938.

Biopsies were taken from a patient who was operated on for genital carcinoma, and who had also kraurotic lesions. The use of 20,000 U of estrin in 20 gm. ointment, massaged in 1-2 times daily during the course of 3 weeks, produced excellent return to normal epithelial conditions, with comfort improved in the first week.—E. L. S.

Tumors of the testicle. Wade, H. K., and F. M. Adams, South. M. J. **31**:496. 1938.

In discussing diagnosis, the value of estimations of prolan A in the urine is emphasized. Prolan A may appear within 3 weeks of the time that swelling is first noticed, and disappear within 2 weeks after total removal of the tumor. The number of units of prolan A per liter varies with the type of the tumor: chorioepithelioma 50,000 or more; embryonal adenocarcinoma 10,000 to 40,000; embryonal carcinoma with lymphoid stroma 2000 to 10,000; seminoma 400 to 2000; and teratoma with adult features 50 to 500.—J. C. D.

HYPOPHYSIS

The action of merthiolate on the gonadotropic effect of anterior pituitary extract. Chen, G., and H. B. van Dyke, J. Pharmacol. & Exper. Therap. **62**:333. 1938.

The gonadotropic effects of sheep pituitary extracts were potentiated by the simultaneous administration of merthiolate (sodium ethyl mercuri thiosalicylate). Merthiolate, administered by itself or at a different site when the anterior pituitary extract was administered, had no effect. The sodium salts of thiosalicylic acid, acetylsalicylic acid and salicylic acid caused no potentiation of the gonadotropic effect. Merthiolate had no effect on the ovary—or testis—stimulating action of prolan. The potentiating effect of merthiolate is due to interference with absorption caused by the chronic inflammatory changes produced.—E. L.

Renal rickets and dwarfism as a pituitary disease. Chown, B., and M. Lee, Amer. J. Dis. Child. **53**:116. 1937.

The authors report a case of a girl of 17½ years who showed the following symptoms: dwarfism, reduced kidney function, polyuria, rickets-like changes in the long bones and delayed sexual development. She had a high normal vitamin D content of the blood and lability of blood calcium and phosphorus. The authors consider that the cause for this syndrome was neither hyperparathyroidism nor chemical deficiency due to renal failure, but a pituitary-diencephalic malfunction.—M. B. G.

The activity of the blood serum amylase in the hypophysectomized dog. Cope, O., A. Hagströmer and Hester Blatt, Am. J. Physiol. **122**:428. 1938.

Changes in the blood serum amylase activity were studied in a series of 7 dogs. In 4 animals which survived total hypophysectomy the activity was doubled and maintained at this higher level. When only the anterior lobe was removed from one dog the activity was likewise increased. A control operation on a pregnant bitch gave results within limits of normal activity. Fasting, glucose, insulin and adrenalin produced only slight changes in either unoperated or operated dogs.—H. W. Schultz.

Studies on experimental hypophysectomy in dogs. III. Somatic, mental and glandular effects. Dandy, W. E., and F. L. Reichert, Bull. Johns Hopkins Hosp. **62**:122. 1938.

Data are presented on the effects of hypophysectomy of 8 puppies as compared with litter-mate controls. Serial sections of the sella demonstrated the completeness of the operative removal of the gland in 6 of the animals. The most striking change observed was the total cessation of skeletal growth soon after total hypophysectomy. The closure of the epiphyseal lines was delayed to 19 months as compared with 7 to 9 months in the controls.—Arthur Grollman.

The calorigenic action of amino acids in the hypophysectomized animal. Evans, H. M., J. M. Luck, R. I. Pencharz and H. C. Stoner, Am. J. Physiol. **122**:533. 1938.

Oral administration of varying amounts of glycine was shown to increase the B.M.R. of both normal and hypophysectomized rats. Ingestion of 0.9% sodium chloride solution elevated the metabolic rate of hypophysectomized rats but depressed it in the normals. The authors believe the increased rate is due to a compensation for an adreno-cortical deficiency resulting from hypophysectomy. Intraperitoneally injected glycine was without effect in either normal or hypophysectomized rats. The B.M.R. for normal rats is given as 783 Cal. per day per sq. m. of body surface (AD = 51); that for hypophysectomized rats 447 (AD = 34). The R.Q. for 59 normals averaged 0.745 (AD = 0.02); that for 41 hypophysectomized rats averaged 0.82 (AD = 0.04). All measurements were made after a 12-hour fast.—H. W. Schultz.

The urea clearance of cats with diabetes insipidus. Farr, L. E., K. Hare and R. A. Phillips, Am. J. Physiol. **122**:288. 1938.

Diabetes insipidus was produced in 6 cats by producing lesions in the supra-optico-hypophyseal tract. Control urea clearances were carried out on 5 normal cats. The average urea clearance for normal cats was 14.3 cc. per sq. m. per min., and for the diabetic cats 13.1 cc. per sq. m. per min. Pitressin decreased the urine volume of the operated cats; it had no effect on the volume of the normal cat. An increased urea clearance was observed in each case, the clearance of the diabetic animal being raised to an average of 16.0 cc. per sq. m. per min. and of the normal to 22.0 cc. per sq. m. per min. Anterior pituitary substance also increased the urea clearances of both groups, the clearance for the diabetic animals being 23.6 cc. per sq. m. per min., and for the normal animal 27.4 cc. per sq. m. per min. There was a tendency for the urea clearance of both the groups to increase with increased urine volumes; it was less marked in the APE experiments than in those involving a usual physiological range of urine flow.—Lena Lewis.

Histophysiology of the anterior lobe of the hypophysis: The anterior lobe and thyroid gland after folliculin injection (Histophysiologie de la préhypophyse. Préhypophyse et glande thyroïde soumises a l'action de la folliculine). Franck, S., Compt. rend. Soc. de Biol. **125**:573. 1937.

Intraperitoneal injections of 20.5 to 30.5 U.S. units of folliculin were given to 4 male guinea pigs. Two hours later, these were killed and the glands examined histologically. Daily subcutaneous injections were given 4 others for periods up to 156 days. In the short

experiment the thyroids were normal, whereas in the longer one they showed signs of increased colloid storage. In the short experiment, the basophils of the anterior lobe were reduced in number, lost their granules, and in some cases showed nuclear degeneration. The longer experiment resulted in similar changes but to a lesser degree. These results support the author's view that the eosinophil cells of the anterior lobe of the hypophysis stimulate the thyroid cells to secrete into the follicle.—J. C. D.

Histophysiology of the anterior lobe of the hypophysis. Anterior lobe and thyroid after treatment with iodine (Histophysiologie de la préhypophyse. Préhypophyse et glande thyroïde soumises a l'action de l'iode). Franck, S., Compt. rend. Soc. de Biol. **125**:569. 1937.

To clarify the part played by the hypophysis in thyroid secretion, 9 adult male guinea pigs were injected daily with anterior-lobe extract for periods of from 9 to 35 days. When thyroid activity had been stimulated, iodine, equivalent to the therapeutic dose used in man, was given by mouth. The hypophyses of the animals receiving anterior-lobe extract alone are known to show a reduction in the number of heavily staining eosinophil and basophil cells and an increase in the small eosinophils and weakly staining elements. The thyroids show evidence of active secretion into the capillaries. In the doubly treated animals of this experiment, the cell relations in the hypophysis approached closely to normal and the thyroids were normal. The author believes that the thyroid cells are stimulated to active secretion into the capillaries by the hormone from the acidophil cells and to secretion into the follicle by that from the basophils. Where the balance between these remains undisturbed, the thyroid is normal. The thyroid activity is, therefore, controlled by the hypophysis.—J. C. D.

The influence of pregnancy on the glucemic response to gonadotropic and prepituitary hormones in the rabbit (Influenza della gravidanza sulla riposta glicemica all'ormone gonadotropo o ad estratti preipofisari nella coniglia). Frada, G., Biochim. e. terap. sper. **25**:71. 1938.

The hyperglucemia following administration of prolan or prepituitary extract, as observed in male and non-pregnant female rabbits, is not obtained in pregnant animals.—A. E. M.

Tolerance and fate of the pressor principle of posterior pituitary extract in anesthetized animals. Larson, E., J. Pharmacol. & Exper. Therap. **62**:346. 1938.

By repeatedly injecting large doses of extracts of pituitary powder into anesthetized cats and dogs, tolerance to the pressor principle was produced. This tolerance was due to inability of the vascular system to undergo further constriction, and not to dilatation of the kidney and muscles or decrease in heart rate. The pressor responses to pituitary extract were increased after sectioning the vagi and denervating the carotid sinuses. When extracts of 3 to 8 mg. of pituitary powder per kg. of body weight were injected into cats or dogs, the pressor principle was eliminated in the urine. The amounts eliminated averaged 25% or less. In dogs the elimination was more rapid than in cats. Incubation of pituitary extract with liver, kidney, muscle or blood of cats, dogs or rabbits under the proper conditions for enzyme activity, causes a disappearance of the pressor principle. Separation studies with the tissue ereptases indicate that amino-polypeptidase is the inactivator.—E. L.

Endoneural secretion of the hypophysis and the neuroendocrine process in general (La neurocrinie hypophysaire et les processus neurocrines en général). Roussy, G. W., and M. Mosinger, Ann. d'anat. path. **14**:165. 1937.

A summarizing article is offered on the significance of migration of cells and the presence of pigment and colloid in the neural lobe of the hypophysis and hypothalamus as evidence of different modes of secretion by these structures. Various ways in which these operate on the vegetative centers in the hypothalamus, extra-pyramidal system of the subthalamus and secretory centers proper are discussed. This hypophyseal mechanism is considered as a specific example of a general endocrine process which involves hormones acting in some situations centrally and in others on the peripheral nerve terminals. One gets the impression that too much weight is attached to histological appearances whose functional significance has not been sufficiently established or which may be artifacts.—A. T. R.

The effects of hypophysectomy and of anterior pituitary extracts on the disposition of fed carbohydrate in rats. Russell, Jane A., Am. J. Physiol. **121**:755. 1938.

The question which these experiments were designed to answer was two-fold: *a)*, does the effect on carbohydrate metabolism, demonstrable in fasted hypophysectomized rats, also

appear in fed animals, *b)*, is the effect of anterior pituitary extracts the same in fed and fasted hypophysectomized rats. Experiments were carried out on approximately 75 young male rats weighing 150-180 gm. Normal and hypophysectomized rats were fasted 24 and 18 hr. respectively. In the case of operated animals tests were made 1-3 months after operation. For injections, standard alkaline extract of beef anterior pituitary was given intraperitoneally in amounts of 1 cc./day and 1 cc. 1-3 hr. before the experiment. Carbohydrate was administered in the form of 5 cc. 25-40% glucose solution by stomach tube without anesthesia. Tissue and blood samples were taken with the animals under amytal. Liver and muscle glycogen, blood glucose, total nitrogen in urine, O_2 consumption, and non-protein R.Qs were determined. Metabolism experiments were done over a 3 hr.-period in a 7-unit closed circuit apparatus designed by M. Kleiber. In the hypophysectomized rats, the proportion of absorbed glucose oxidized and of total calories obtained from carbohydrate were both higher than in normal rats. Decreases in the amount of glycogen stored were accounted for by increases in the rate of oxidation of carbohydrate. The injection of anterior lobe extracts in both hypophysectomized and normal rats caused an immediate fall in the apparent rate of oxidation of carbohydrate after this substance was fed. In the hypophysectomized rats the level was brought below normal. More carbohydrate was stored as glycogen or as blood and tissue sugar by the injected rats. Normal rats injected with anterior lobe extract for 20 days became entirely resistant to the usual action of the extracts in suppressing carbohydrate oxidation and increasing storage after feeding. Normal, unfed rats, in which carbohydrate oxidation was apparently already at a low level, showed no change in R.Q. or carbohydrate levels when treated with the pituitary preparation. Hypophysectomized rats unfasted (which had ample carbohydrate supplies) had much higher R.Qs than did normal animals under the same conditions. It was concluded that the anterior pituitary is concerned not only with preservation of carbohydrate during fasting, but also with it disposition when it is fed.—K. A. Brownell.

Effects of hypophyseal implants on extremities of salamander larvae (Die Wirkung der Implantation zusätzlicher Hypophysen auf die Molchextremitäten). Schaefer, K., Arch. f. Entwcklngsmechn. d. Organ. **136:**563. 1937.

Whole hypophyses (8 to 10) were implanted (2 or 3 at a time) into 4 salamander larvae. Grossly, there resulted increased epidermal mucus, ridging of the skin, penetration of bones through the skin, and disappearance of joints and ultimately of entire digits. Histologically there was increased growth of epiphyseal cartilage, necrosis of metaphyseal cartilage with separation of epiphysis from diaphysis and expulsion of the latter. Muscles and nerves degenerated. The skin became very edematous and the epidermis vacuolated. These degenerative and myxedematoid changes are interpreted as being due to hypofunction of the thyroid gland.—A. T. R.

Post-partum necrosis of the anterior pituitary. Sheehan, H. L., Edinburgh M. J. **45:**(Tr. Edinburgh Obst. Soc.) 13. 1938.

In 16 cases, of which all patients died 14 or more hours postpartum after severe hemorrhage and collapse, 10 cases showing necrosis of the pituitary. In 50 cases which recovered from severe postpartum hemorrhage, 15 showed evidence of pituitary disfunction. The author suggests that there is normally a reduction of the flow of the blood through the pituitary at the termination of pregnancy. In cases of severe hemorrhage, the flow of blood may be further reduced until there is stasis in the gland, with the possibility of thrombosis and subsequent glandular necrosis. This would account for the pathological picture in the deceased, and explain the frequency of pituitary dysfunction in those recovered.—J. C. D.

The use of pigeons as test animals for a hormone of the anterior lobe of the hypophysis (Sur l'utilization du pigeon comme animal test d'une hormone préhypophysaire). Valle, J. R., Compt. rend. Soc. de Biol. **125:**687. 1937.

When the crop gland is used as a test for prolactin, this involves killing the bird. The author describes his method of inserting a window in the throat, so that the changes in the gland may be observed in the living bird.—J. C. D.

Periodic discharge of the hypophyseal hormones by the arteries (Über die Abscheidung von Hypophysenwirkstoffen auf dem Arterienweg im Rahmen einer periodischen Tätigkeit der Hypophyse). Winterstein, J., Ztschr. f. Anat. u. Entwcklngsgesch. **107:** 427. 1937.

A histological study of serial sections of 3 adult human hypophyses and adjacent hypothalamus was made with special reference to the blood vessels as a means of expelling the

hormones. The connective tissue framework of the hypophysis is regarded as contractible and together with the vascular system may be a pumping mechanism for blood and colloid. Squamous-like cells, often found near blood vessels, may have some functional connections with the change in caliber of the vessels.—A. T. R.

PANCREAS

The pancreas and the deposition of fat in the liver. Best, C. H. and Jessie H. Ridout, Am. J. Physiol. **122**:67. 1938.

Previous experiments indicating that the pancreas contained no new specific substance which affects liver fat in white rats were further substantiated. Experiments using large groups of rats, in which fresh beef pancreas, liver and muscle were added to a basal diet, showed only lipotropic effect which was predicted from its choline and protein content. Similar results were found using a pancreatic extract prepared by the Dragstedt method, an extract reported to have a potent lipotropic effect on depancreatized dogs.—K. P. McConnell.

The action of insulin on glycogen reserves. Bridge, E. M., Bull. Johns Hopkins Hosp. **62**:408. 1938.

Normal unanesthetized rabbits were infused at a constant rate with glucose and insulin. The predominant effect of the insulin was to cause a shift of the glycogen deposition from the liver to the muscles.—Arthur Grollman.

Chromaffine and argentaffine cells in the pancreas of man and mammals (Cellule enterocromaffini e cellule argentofile nel pancreas dell' uomo e dei mammiferi). Erspamer, V., Ztschr. f. Anat. u. Entwcklngsgesch. **107**:574. 1937.

Cells with the chromaffin reaction were found singly or in groups along the pancreatic ducts of the 22 species studied. In the human pancreas they were found only in the extraduodenal part of the major and minor pancreatic ducts. In some species such cells were found among the pancreatic alveoli and even in the island of Langerhans. In the pancreas of all the species studied cells with argyophil granules were also found. These cells are thought to be immature chromaffine cells and endocrine in nature, but not island cells.—A. T. R.

Note on insulin tannate (Sur l'insuline-tannate). Lun, F., Compt. rend. Soc. de Biol. **125**:1088. 1937.

In rabbits, insulin mixed with equal parts of a 2% solution of tannic acid had a more prolonged hypoglycemic action than pure insulin. If 10% of a 1/200 normal solution of sodium thiosulfate was added to this, the action of the insulin was reduced and its effects still further prolonged.—J. C. D.

Congenital aplasia of the islands of Langerhans with diabetes mellitus. Moore, R. A., Amer. J. Dis. Child. **52**:627. 1936.

The author reports a case of congenital diabetes associated with aplasia of the islands of Langerhans in a girl of 13 years. He states that a decrease in the number of islands in diabetes is a well-established finding, and occurs in about 20% of all cases. Absence of the islands has not been reported since the early studies of Szoboluv in 1902 and Dieckhoff in 1895. Congenital hypoplasia of the pancreas as a whole is rare.—M. B. G.

Changes in blood lipids during insulin treatment of schizophrenia. Randall, L. O., D. E. Cameron and J. M. Looney, Am. J. M. Sc. **195**:808. 1938.

In 5 schizophrenic patients, who showed a good remission during the insulin hypoglycemic treatment, a significant rise in whole blood phospholipid, total cholesterol, and total lipid occurred. In 9 of 11 patients who did not improve during the treatment, a mild lipemia occurred during the early weeks of treatment, followed by random variations about the initial premedication mean. Insulin did not significantly affect the post-absorptive level of the blood lipids.—Author's Abstract.